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PRESIDENTIAL ADDRESS

PLANT VIRUS RESEARCH IN INDIA*

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Virus diseases of plants are responsible for serious losses to our agricultural crops. They are all the more important in the plantation crops as also those which are propagated vegetatively. Ever since the discovery of the tobacco mosaic virus in 1892, over 300 different viruses attacking plants have been described. Although accurate figures of losses due to these are not available it is now known that the damage they cause probably equals that due to all other disease-causing agents.

Curly top of sugarbeet almost threatened the existence of sugarbeet in the United States of America from 1916 to 1932 and is still found to cause severe damage to a number of other crop plants. More than 18 virus diseases have been reported on peach alone which have destroyed thousands of trees rendering peach-growing impossible over large areas. Tristeza or quick decline disease of citrus is known to be present in almost every citrus growing country of the world. It causes heaviest losses to citrus trees of certain scion-stock combinations notably those of Sweet Orange budded on Sour Orange and some other root-stocks. Within 12 years after the disease appeared in one of the states of Brazil, about 75% of the trees of the state had been destroyed. In Great Britain, the greatest losses caused by virus diseases are in the potato and sugarbeet. In West Africa the existence of cacao plantations is being threatened by the swollen shoot complex of viruses. In the Gold Coast its production has been reduced by about 50 per cent. The loss in annual crops depends greatly on the time at which the plants get infected and infection in early stages of plant growth may mean no crop. The losses in plantation crops which begin to bear after several years of careful looking after, however, mean very heavy financial commitments for the growers.

This group of diseases, because of their economic importance, has received due attention in other countries and the science of Virology has made considerable progress. The most outstanding advances made are the isolation and crystallisation of several viruses,studies of their chemical composition, and the biochemical studies arising out of these. The purification of plant viruses which was originally effected by the drastic chemical methods has been replaced by the less harmful high speed centrifugation method. Great improvements have been made in the high speed centrifuges, and refrigerated Ultra-centrifuges are now being used in the isolation of viruses and study of their physical properties. More attention is being paid now to the study of properties of the purified virus preparations which have helped a great deal in the proper understanding of the nature and properties of the viruses. The new inventions have made possible direct study of the shape and size of the virus particles and also

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the molecular arrangements on the faces of the virus crystals so that viruses can now be direct.y seen and their electron micrographs taken.

The serology of plant viruses which had received little attention in the past has now developed into an important branch of virus study. It has been possible now to differentiate the virus strains by the use of serological reactions and this method is now considered sound for identifying viruses and virus strains. Serological reactions also help in the quantitative assay of the viruses during multiplication or inactivation. They have also found use in the quick detection of the viruses in certain crop plants and ensuring their freedom from virus infection.

Investigations have also been carried out on the relationship of viruses and their insect vectors. Most of the the earlier work in this field was limited to finding out the vectors of the virus diseases of important crop plants. During recent years attempts have been made to study the mechanism of insect transmission. Evidence of hereditary transmission of viruses in insects and the multiplication of the viruses in the insect bodies has been obtained. Techniques have been devised to directly inoculate the insects by injecting infective insect juices and to get direct evidence of the multiplication of the viruses in insects through serial transfers. A variety of new species of insects such as mealy bugs and mites have been shown to be the vectors of virus diseases in recent years so that new and more potent vectors are being established.

As the knowledge about the plant viruses is rapidly increasing, new techniques or procedures are being devised to replace the existing methods of control. While the seed certification and the use of virus-free clones of important fruit and vegetatively propagated crops is receiving wider application, the use of heat and chemotherapeutic treatments has been extended to a number of virus diseases of fruit and other cash crops e.g. peach, strawberry, raspberries and sugarcane. The possibilities of the use of antibiotics in the control of plant virus diseases are being explored. The recent introduction of the systemic insecticides has opened new opportunities for the direct control of the insect vectors. Study of this aspect is of basic importance in a country which is primarily agricultural.

The biological control of plant viruses has been the most neglected subject till to-day although it appears to offer considerable scope. It is well known that several fungi and viruses parasitise the insects and this fact could be exploited in the interests of the agriculturist. It would, therefore, be of interest to study the fungal and virus diseases of insects, particularly the vectors, with a view to explore the possibilities of biological control.

It has been observed that the presence of one virus in plant tissues precludes entrance of a related virus. This type of non-sterile immunity allows the possibility of inoculating a plant with a mild or attenuated strain of a virus with a view to protect it against infection with the severe strains. For protective inoculation or the so-called vaccination of plants, there are two essential requirements; first, a number of strains of the virus must be available, and secondly, one of the strains must be an avirulent

one so that the development of milder strains and possibility of their production by artificial mutagenic agents deserves attention.

Systematic work on virus diseases of plants in India was started only about twenty years back. During this period several diseases of economic crop plants, fruits and vegetables as well as plantation crops have been described in order to seek methods whereby the viruses spread in nature, and the variety of plants they infect, with a view to evolve suitable methods of their control. Among the diseases of vegetables, potato appears to have received somewhat greater attention in view of the fact that there was complete closing down of the large importations of foreign seed potatoes during the Second World War, and the general deterioration in quality of the local seed on account of the 'running out' of the seed stocks due to virus diseases. As a result of systematic investigations carried out at the Indian Agricultural Research Institute, it has been shown that a number of viruses are commonly associated with our commercial stocks. As the virus diseases affecting potato crop are tuber perpetuated, the only method of combating them is the production of nucleus disease-free seed stocks. Any programme directed for the improvement of potato must, therefore, chiefly involve the disease aspect and certification therefrom. The Indian Agricultural Research Institute has been the pioneer in producing virus-free seed potatoes of important varieties in India. As a result of mass selection, indexing and testing on differentials, virus-free nucleus seed stocks of potato varieties *Darjeeling Red Round, Up-to-date* and many promising hybrids were produced under a scheme of certification in order to put the potato industry on sound footing. In addition, as an interim measure to step up potato production, partly disease–free seed potatoes were produced in large quantities by systematic roguing in cultivator's fields and made available to the growers. The performance of such seed was found to be very satisfactory both from the point of view of disease incidence and out-turn. The nucleus seed stocks were transferred to the newly established Potato Institute for multiplication and distribution to the growers. The future of potato industry depends on how fast we can succeed in putting certification programme into practice, as this is the only reliable method by which yields have been increased two-folds in some of the advanced countries. It is regrettable that in spite of the fact that work in India has shown great possibilities in this direction and that yields could be easily stepped up, the certification system, nucleus of which was established under a scheme financed by the Indian Council of Agricultural Research during the war, has not even till today taken a practical shape. Seed certification though it has to be established on all-India basis should form the responsibility of the State Departments of Agriculture. The centre could only help in laying down the standards but selection of varieties as also the multiplication of nucleus seed stocks and distribution would have to be done directly by the States if the practical objective has to be achieved.

Virus diseases of other Solanaceous crops have also received attention. *Bemisia tabaci*, vector of tomato leaf-curl, which has wide host range has since then been found to be a potent vector of a number of virus diseases in India and has been shown to carry more than one virus simultaneously.

Yellow vein mosaic of *Abelmoschus esculentus* is a serious problem wherever *Bhindi* is grown. It has resulted in failure of the crop when infection has been particularly early. No cultivated variety has shown resistance to the disease. However, some wild species such as *Abelmoschus manihot* var. *pungens* and *A. crinitus* have been found to be immune to infection with the virus. Attempts are being made to utilise these sources of resistance for production of suitable resistant varieties.

The study of virus diseases of fruits which is of basic importance to the development of horticulture has been only recently taken up on a systematic basis. Bunchy top of banana has been found to be responsible for severe losses to the banana industry in the South. The disease is believed to have been introduced from Ceylon in 1940 and is widespread in Kerela State. A mosaic disease has recently been shown to be widely prevalent in the Bombay State and is transmitted by *Aphis gossypii*. As these diseases are restricted to certain parts of the country, strict plant quarantine measures are necessary to prevent their spread to the large areas of the country where these diseases have not yet obtained a foothold. In the areas of their occurrence, however, use of virus-free suckers and destruction of the sources of infection are the only feasible methods at present.

Papaya mosaic transmitted by *Aphis gossypii, A. malvae, A. medicaginis* and *Myzus persicae* is taking a heavy toll of the crop in the states of Bombay, Bihar, Bengal and Madhya Pradesh. So far no varieties appear to show resistance to the disease. Some exotic material of *Carica* spp. imported recently from abroad is being tested with a view to locate sources of resistance. Leaf-curl, on the other hand, generally appears to be common in the nursery stocks. The plants appear to differ in their reaction to the disease at different ages.

The work on decline disease of citrus characterised by pronounced veinal chlorosis, chlorotic spotting and twig decline has revealed that the disease in India is also of virus origin and is transmitted by the citrus aphid, *Toxoptera citricidus*. The virus has been transmitted by tissue grafting to Standard Sour Lemon, Sour Orange and West Indian or Key lime producing symptoms similar to those observed for tristeza group of viruses.

The study of virus diseases of stone fruits has been taken up recently. 'Liné Pattern' of plum, plum mosaic, marble disease of cherry, mosaic of *Rubus ellipticus*, peach mosaic, variegated mosaic of apple, 'line pattern' of almond etc. are some of the virus diseases recorded so far. Stone fruits are generally grown in sections affording suitable cultural factors. Virus diseases affecting stone fruits occur in all sections but they are most prevalent in those ones where a large number of different kinds of these fruits are associated. Since some of these diseases are known to have rapid rates of spread it would, perhaps, be desirable to carry out investigations generally in the regions of their occurrence. Although heat and chemotherapeutic treatments have been effectively used for the control of certain stone fruit viruses, the only practicable method of combating such di-

seases is the production of virus-free nursery stocks and their distribution under a recognised system of certification. The certification programme has to be accomplished in two stages. In the first stage the production of virus-free nucleus nursery stocks is to be achieved by proper selection of apparently healthy fruit trees, testing them on differentials for freedom from virus infection and their further propagation under controlled insect-proof conditions. The next step is to multiply the virus-free nucleus stocks in sufficient quantities to meet the needs of growers.

Under cash crops, virus diseases of sugarcane, cardamom and cotton deserve special mention. Three strains of mosaic of sugarcane have been described from this country and a constant watch on the occurrence of more virulent strains requires to be maintained if we have to continue to grow this crop successfully. "Grassy shoot" a serious disease of sugarcane has recently come to light which appears to be assuming alarming proportions and may become a limiting factor in cane cultivation if proper measures of control are not forth-coming in time. The disease has been shown to be transmitted by *Longiunguis sacchari*, *L. pseudosacchari* and *Aphis maidis*' and *Jowar* (*Sorghum vulgare*) has been found to be the alternative host of the virus. 'Katte' disease of small cardamom and 'Foorkey' of large cardamom have also been studied and their insect vector and alternative hosts reported. A complete and effective control of 'Katte' disease has been achieved in the North Kanara district of Bombay State by roguing of diseased plants and rehabilitation of the plantations with healthy stocks. Varietal resistance tests with 'stenosis' or 'small leaf' disease of cotton have shown that some Indo-American hybrids are immune to the virus. It is not yet known whether this virus is in any way related to the one reported from Punjab which affects only the American cottons.

Recently, a new group of virus diseases which result in phyllody have been found to be widespread in crops like Sesamum and Sannhemp. The causal virus which is transmitted by the jassid, *Deltocephalus* sp., appears to have a very wide host range among the cultivated as well as ornamental plants.

Although a large number of problems of virus nature concerning a variety of crop plants, vegetables and fruits have been studied to near satisfaction in the past there are a number of problems in this subcontinent which still remain unsolved. Among such problems of national importance mention may be made of the mango malformation disease, which is fast spreading in most of the mango-growing areas of the country. The observations so far recorded lead us to believe that the disease is of virus origin. The spike disease of sandalwood which is widespread in Madras, Mysore and Coorg is responsible for severe losses to the sandalwood industry of the country. The method of transmission of the virus in nature and the insect vector have not yet been established and require careful research. Also, possibility of the presence of variety of alternative hosts and symptomless carriers cannot in this case be ruled out. The problem needs concerted effort by experienced virus specialists if we have to save this valuable industry for the country.

The root disease of coconut which has seriously affected the economy of the coconut growing areas of the country needs to be studied on systematic basis. The disease resembles in certain respects the one described from Philippines in as much as the yellowing or bronzing of outer whorls of leaves, retardation in production of female flowers and bearing etc. The flaccidity of leaves observed in infected Indian palms, however, does not appear to be of common occurrence in the Philippines. The difference in symptom expression may possibly be connected with environmental conditions. Two forms of wilt i.e. 'Bronze leaf wilt' and 'tapering stem wilt' have been recorded from West Indies, 'Bronze leaf wilt' being serious of the two. A root disease of coconut palm has also been described from New Guinea. Another root disease of coconuts ascribed to physical or physiological drought has been reported from Ceylon. Even though the actual causes of these diseases have not yet been established, their possible relationship cannot be ruled out. The evidence so far available points to the possibility of Indian root disease as also the one in the Philippines being of virus origin. Concerted effort of a group of very experienced scientists is esential if we have to mitigate the losses due to this serious disease in the already over-strained economy of these areas.

Although no regular surveys for the distribution of tristeza group of viruses have so far been carried out in India, nevertheless, the preliminary surveys conducted in several States show that they are widespread and are responsible for extremely heavy losses. In the light of the observations made and the preliminary investigations conducted so far, there are reasons to believe that citrus in India is infested by more than one virus disease and a planned programme for rehabilitation as also control of these diseases requires to be taken up immediately from all angles. It would have to be borne in mind in planning rootstock tests in the infested areas that several standard scion varieties of citrus may carry one or more of these viruses. The study of virus complexes and the modes of dealing with individual viruses would have to be devised. Survey of insect fauna for the determination of vectors and their activity would have to be carried out simultaneously. Investigations on the host range of the virus within Rutaceae and closely allied families as also isolation and identification of strains of the virus and the effect of the individual strains on a variety of plants would also be necessary. Control measures for this serious malady have to be studied from two stand points i.e. the saving of the existing plantings of suscepitible scion-stock combinations and avoiding losses in new citrus plantings. Tolerant stock-scion combinations would have to be determined and it would be necessary to ensure that the budwood employed by the citrus growers is free from virus diseases.

I have attempted to focus attention on gaps in our knowledge and some of the problems that face us. The problems are emergent and have gained importance during recent years. I would, therefore, invite all those interested in the subject to make their contribution in mitigating economic losses. Keeping in view the diversity of the problems, both fundamental and applied, it is obvious that intensive study of plant viruses would have to be taken up at the Agricultural Research Institutions and

the Universities if we have to succeed in saving our food crops from ravages of such diseases, and in raising the standard of living of our people. As far as training in plant viruses is concerned the arrangements at present are far from adequate. It is necessary to provide expert staff for training in this sphere of Plant Pathology in the scientific institutions and colleges. Greater investments in research relating to virus diseases will no doubt lead to greater returns by cutting down losses in our agricultural crops and it is hoped that the administrators would find ways to finance these on the required scale. We, as Plant Pathologists, feel that we are now, in view of the knowledge that has been built up as a result of research for several decades, in a better position than ever before to deal with them adequately.

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SEEDLING AND ADULT PLANT REACTIONS OF WHEAT VARIETY RIDLEY TO STEM RUST

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It is generally believed that a variety of wheat which is resistant to a particular race of a rust in the seedling stage usually remains so to the same race in the adult stage also. On the other hand, a variety susceptible in the seedling stage may or may not remain susceptible in the advanced stages of growth. On the basis of these observations Goulden, Newton and Brown (1930) distinguished 3 groups of wheat varieties based on the field (adult stage) and glasshouse performance in respect to *Puccinia graminis tritici*. The first group of varieties which included Khapli (Indian Emmer), Vernal, Quality etc., were resistant both under field and glasshouse conditions. The second group comprising of varieties like Pented, Acme and Hope H44–24 displayed significant differences in rust reaction in the seedling and mature stage. The third and the last category included wheat varieties like Reward, Kota and Marquillo where plants, on an average, exhibited more resistance in adult stage than in the seedling. Johnston (1937) has shown that with advancement of age plants developed a kind of resistance which he called as "maturative type" of resistance seen, for example, in Tenmarq wheat. Many workers such as Stakman and Piemeisal (1917), Rudrof and Job (1934), Johnston and Mains (1932) etc. have demonstrated that plants susceptible in the seedling stage showed a marked resistance in the adult stage. Based on a large number of reports Scheibe (1930) has concluded that in case of leaf rust no change in the reaction with age should be expected if the variety is very resistant or very susceptible "while change from seedling susceptibilty to mature plant resistance is found only in varieties that exhibit a moderate degree of susceptibility or intermediate rust reactions" (Chester - 1946).

This rule, however, has not got universal application and in some cases variety found resistant in the seedling stage does become susceptible in the adult stage. Such cases, however, are few. Campos, Gibler and Borlaug (1953) recorded that in many crosses, involving variety Mentana, resistant seedlings, many a time, become susceptible in the adult stage. They have also concluded that the phenomenon of seedling resistance and adult susceptibility is by no means unique in crosses involving Mentana wheat alone. In 1952-53 they studied more than 200 crosses and recorded that 34 of these exhibited this phenomenon and many of these lines did not have Mentana as a parent. A phenomenon similar to that observed by Campos *et al* (l.c.) has been noticed in case of wheat variety Ridley. This variety received from Australia has been found to be resistant, in the seedling stage, to most of the races of black rust met with in India and has been released for cultivation in the hilly regions of North India. On the whole the variety is still doing well in those regions. It has been

found that at times it gets infected by those very races of black rust to which it is resistant in the seedling stage. Complete breakdown of this variety has, however, not been reported so far.

For investigating the reasons for the breakdown of variety in advanced stages of growth, plants were raised in 10″ pots and kept outside till the middle of January with a view to provide as far as possible natural conditions. Since black rust appears in Delhi sometimes by the end of January or beginning of February it was not considered desirable to keep the plants outside after the first week of January and they were transferred to spore proof glasshouse, and inoculated after the emergence of ears by the end of February or beginning of March.

For inoculation the plants were kept in a humid chamber for nearly six hours before and for 48 hours after inoculations. To ensure uniform distribution of uredospores the inoculations were done by dusting the uredospores with the help of a blower. For this purpose Talc was mixed with uredospore dust in the ratio of 1 : 200 and this mixture was gently released in the chamber so that it gradually settled down uniformally on all parts of the plants. Forty eight hours after the inoculations the plants were transferred to glasshouse benches. Along with these plants, seedlings of Ridley raised in 4″ pots and with only the first leaf fully unfolded, were also inoculated in the same chambers. Since seedlings and adult plants were inoculated under identical conditions all other factors such as light, humidity, temperature etc. could not have been in any way responsible for the variation in reactions. In a few cases, besides the seedlings of Ridley, full grown plants of Agra local wheat, a highly susceptible indigenous variety of *Triticum aestivum* L. were also kept for the sake of comparison of reactions of the adult plants.

The results of seedling as well as adult resistance tests against races 15, 15-C, 21, 21-A, 40, 75, 117, 122 and 194 of black rust are given in the table.

It is observed from the data presented that the variety is susceptible to only races 15–C and 122 in the seedling stage (on the basis of first leaf reaction) and resistant to all the remaining ones. In the adult stage, however, the stem, sheath and awns were found to develop susceptible type of pustules even with those races to which it was resistant in the seedling stage. (Plate 1). In case of leaves, however, only resistant type of pustules appeared and in no case clear cut susceptible type of pustules were observed. In this respect different leaves of Ridley plants varying from 1 to 3 months in age as well as newly developed leaves hardly 10 days old did not reveal any appreciable differences in rust reactions. Age of the leaves, therefore, does not appear to have any relation with the rust reaction in this particular case. No variation due to age of the leaf, as has been recorded by Johnston and Melchers (1929), Scheibe (1930) etc. has been observed during the course of these experiments. The case of the other parts of the plants, such as stem and awns, was, however, different and these parts developed typical susceptible type of pustules as appeared in the adult plants of a susceptible variety, Agra local wheat (an indigenous variety of *Triticum aestivum* L.) maintained as control.

Reactions of wheat variety Ridley (E. 572) in the seedling and adult stages to races 15, 15–C, 21, 21–A, 40, 75, 117, 122 & 194 of *Puccinia graminis tritici*.

Races	15	15–C	21	21–A	40	75	117	122	194
No. infected / No. inoculated	12 / 12	10 / 10	0 / 12	0 / 14	0 / 15	11 / 11	0 / 10	14 / 14	10 / 10
Seedling reactions	1–2	3–4	0;	0;	0;	0;–1	0;	3	0;–1
Adult Plant Reactions:—									
Stem & sheath	Suscept.* type of pustules	Suscept.* type of pustules	Small* suscept. type of pustules	Suscept.* type of pustules	Suscept.* type of pustules	Suscept.* type of pustules	Suscept.* type of pustules mainly on nodes	Suscept.* type of pustules	Suscept.* type of pustules
Awns	Few* suscept. type of pustules	Suscept.* type of pustules	Small but suscept. type of pustules	Small suscept. type of pustules	In conclusive	Few* suscept. type of pustules	Few* suscept. type of pustules	Suscept.* type of pustules	Suscept.* type of pustules
Leaves	Few scattered rest. type of pustules	Suscept. type of pustules	Small resist. type of pustules	Small resist. type of pustules	Few small resist. type of pustules	In conclusive.	Small resist. type of pustules	Small suscept. type of pustules	In conclusive.

*Susceptible type of pustules were isolated, analysed and races with which plants were inoculated were recovered.

Leaf and Stems of Wheat Variety Ridley inoculated with race 21 of *Puccinia graminis tritici*.

Summary

Ridley variety of wheat, which is resistant in the first-leaf stage to races 15,21, 21-A, 40, 75, 117 and 194 of black rust, shows different degrees of resistance in different parts of the plant in adult stage. While the leaves of adult plants of different ages were resistant to races 15,21,21-A, 40 and 117, the stem (particularly above the nodes), leaf-sheath and awns showed long, coalescent, susceptible type of pustules. With races 75 and 194, however, reactions on the leaves were inconclusive though susceptible type of pustules developed on the stem, leaf-sheath and awns.

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*Originals not seen.

UEBER EINE ERKRANKUNG AN TAXUS IM HIMALAYA

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Eine gemeinsame Exkursion nach Ostgarhwal im Zentralhimalaya, welche für den erstgenannten Autor durch eine Reisestipendium der Schweizerischen Naturforschenden Gesellschaft ermöglicht wurde, gab uns Gelegenheit parasitische Pilze aus verschiedenen Gruppen zu sammeln. Unsere Reise wurde auch grosszügig unterstützt vom Forest Department U. P. In uneigenütziger Weise wurden uns die Rest Houses und Unterkunftshütten zur Verfügung gestellt und wir konnten in jeder Beziehung auf eine tatkräftige Hilfe zählen. Es ist uns deshalb ein Bedürfnis, Herrn Konservator Jain (Naini Tal) und Range Officer Shri Tej Sigh Negi für all ihre Hilfe zu danken. Ihnen sei die vorliegende Arbeit gewidmet.

Taxus baccata L. zerfällt nach Pilger (1903) in mehrere Subspecies, von denen *Taxus baccata* L. subsp. Wallichiana (Zucc.) Pilger [*Taxus Wallichiana* Zucc.] die südostasiatischen Gebirge vom Himalaya bis zu den Phillippinen besiedelt. Es sind denn auch Pflanzen dieser Subspecies, welche wir in höhern Lagen, eingestreut in den Quercus-Wald [*Quercus semicarpifolia* Sm.] angetroffen haben.

Aber die Bäume waren im durchwanderten Gebiet krank. Von weitem fielen sie auf durch gelbe Verfärbungen an den Nadeln. Die Untersuchung des mitgenommenen Materials ergab, dass auf den kranken Nadeln zwei Pilze vorkommen, welche beide für die Erscheinung verantwortlich sein können:

In erster Linie sind uns die dunklen Rasen einer Meliolaceae aufgefallen. Auf den dunkelbraunen Myzelpolstern des Pilzes konnten wir auch zahlreiche Fruchtkörper in verschiedenen Entwicklungsstadien feststellen, sodass einer genauen Untersuchung nichts im Wege stand. Der Pilz lässt sich folgendermassen beschreiben:

Die dunkelbraunen Myzelrasen sind rundlich oder meist in der Substratrichtung etwas gestreckt, 1 bis 3 mm lang und 1/2 bis 1½ mm breit. Sie setzen sich aus 5–6 μ dicken, kurzgliederigen, reich verästelten und oftmals gekrümmten, oberflächlichen Hyphen zusammen. An diesen sitzen zahlreiche kugelige oder undeutlich ellipsoidische, selten auch schwach keulige, 10–13μ grosse Hyphopodien, welche mit einer kurzen Trägerzelle am Myzel sitzen. Borsten fehlen und wir konnten auch keine Konidien feststellen. Im Zentrum der Rasen beobachtet man häufig, aber nicht immer kugelige, an der Basis mit zahlreichen dunklen Hyphen mit dem oberflächlichen Myzel verbundenen, 130–170μ grosse Fruchtkörper. Diese sind sehr dickwandig und völlig geschlossen. Aussen ist die Wand unregelmässig mit stumpf kegelförmigen oder dornigen, aus mehreren sehr dunklen Zellen zusammengesetzten Höckern bewehrt und sie baut

sich aus mehr oder weniger derbwandigen, 8–15 μ grossen, regelmässig vieleckigen, braunen Zellen auf. Die Fruchtkörperhöhlung ist mit wenigen Lagen von sehr hellen, zartwandigen, stark zusammengepressten Zellen ausgekleidet.

Die zartwandigen, zylinderischen Asci reifen nicht gleichzeitig. Bei der Reife lösen sie sich schleimig auf, kurz vorher messen sie 90–100x 18–25 μ : sie sind zweisporig und von 3–5 μ breiten, zellig–fädigen, Paraphysen von verschiedener Länge umgeben. Die Ascosporen sind breit spindelig, beidendig breit abgerundet, 41–51 x 15–18 μ gross, opak schwarzbraun und durch 3 Querwände vierzellig. An jeder Querwand sind sie deutlich eingeschnürt und die beiden mittleren Zellen sind deutlich länger als die Endzellen. (Abbildung 1.)

In der monographischen Bearbeitung von Stevens (1927) sind auf Taxaceae zwei Meliolaceae angegeben, mit welchen der vorliegende Pilz verglichen werden muss. Es handelt sich um zwei sich nahe stehende Arten aus der Gattung *Irenina* Stev., nämlich *Irenina pitya* (Sacc.) Stev., beschrieben von *Taxus canadiensis* Marsh. [=*Taxus baccata* L. subsp. *canadiensis* (Marsh.) Pilger] und *Irenina podocarpi* (Doidge) Stev. beschrieben von Podocarpusarten aus Südafrika. *Irenina pitya* zeichnet sich durch gelappte Hyphopodien aus, die Sporen sind aber wie auch bei *Irenina podocarpi* mit unserer Form aus dem Himalaya übereinstimmend gebaut. Neuerdings ist von Sawada (1950) eine dritte Art, *Irenina taxi* beschrieben worden, welche auf *Taxus baccata* L. subsp. *cuspidata* (Sieb. et Zucc.) Pilger [=*Taxus cuspidata* Sieb. et Zucc.] parasitiert. *Taxus baccata* subsp. *cuspidata* steht der Form im Himalaya recht nahe, kommt aber in Japan vor. Nach der Beschreibung kann unser Pilz mit *Irenina taxi* Sawada identifiziert werden.

Hansford (1956) hat nun allerdings festgestellt, dass die Gattung *Irenina* Stev. mit *Asteridiella* McAlpine zusammenfällt er schlägt denn auch für alle als *Irenina* beschriebenen Pilze die entsprechenden Neukombinationen vor. Unser Pilz heisst demnach *Asteridiella taxi* (Sawada) Hansf. [Leider gibt Hansford, 1956, fälschlicherweise Stev. als Klammerautor an].

Der zweite auf dem Material gefundene Parasit gehört in die Nähe von *Chaetothyrium* besitzt aber zweizellige, hyaline Sporen. Für derartige Pilze wurde die Gattung *Chaetothyrina* Theiss. aufgestellt. Die vorliegende Form stellt offensichtlich eine neue Art dar, die wir zu Ehren unseres stets hilfsbereiten Begleiters Shri Tej Singh Negi *Chaetothyrina Negii* nennen möchten. Die Art lässt sich folgendermassen beschreiben:

Chaetothyrina Negii nov. spec.

Plagulae effusae, superficialiae, plus minusve minutae, hyphis olivaceo-fuscis, 3–4 μ crassis, curvatis, ramosis compositae. Hyphopodia, setae et conidia nulla. Perithecia solitária 100–120 μ diam. 45–55 μ altitudine, setis opaco-fuscis, usque ad 80 μ longis et basialiter 4–5 μ crassis ornata, poro apicali irregulari. Pariete peritheciorum extus cellulis mäandricis, fuscis compositus, intus hyalinus, cellulis plus minusve compressis compositus.

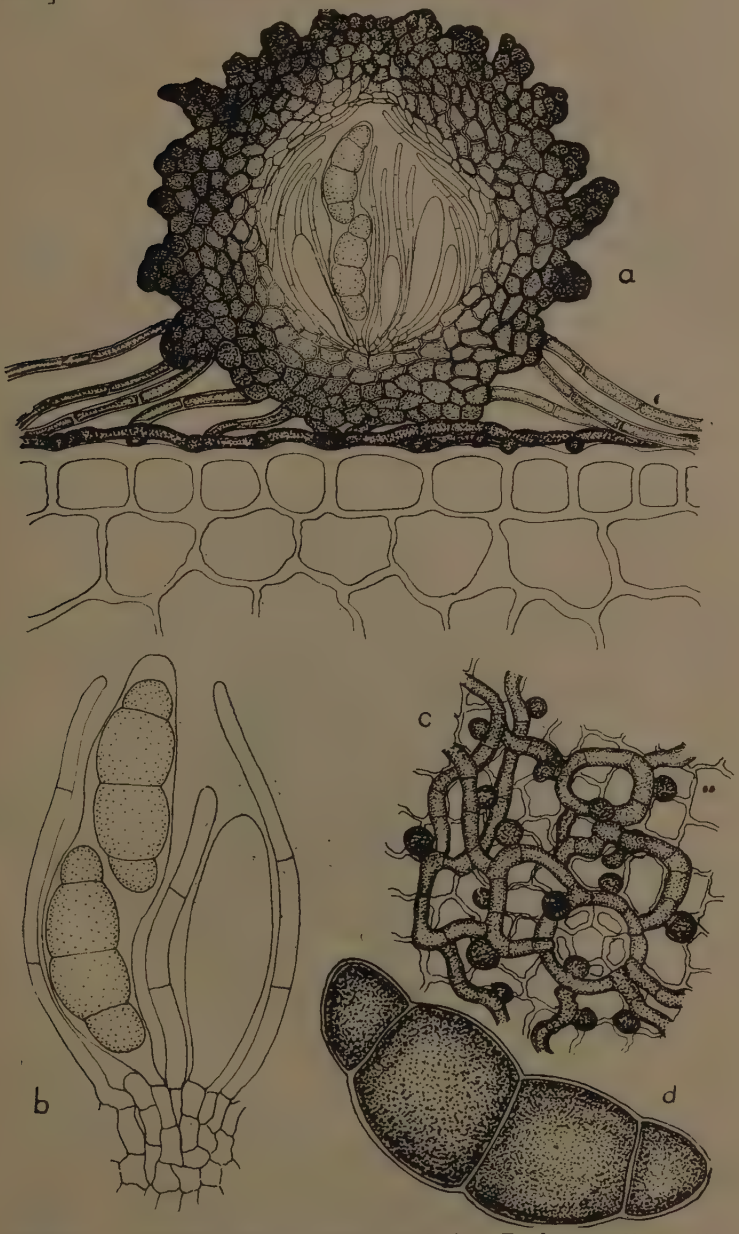

Abbildung 1. *Asteridiella axi* (a) Schnitt durch einen Fruchtkörper Vergr. 250 x; (b)Asci Vergr. 5000 x; (c)Myzel Vergr. 250 x; (d)Ascospore Vergr. 1000 x.

Plate I. *Asteridiella taxi* (a) Section through a fruit body Magnification 250 x; (b) Asci Magnification 5000 x; (c) Mycelium Magnification 250 x; (d) Ascospore Magnification 1000 x.

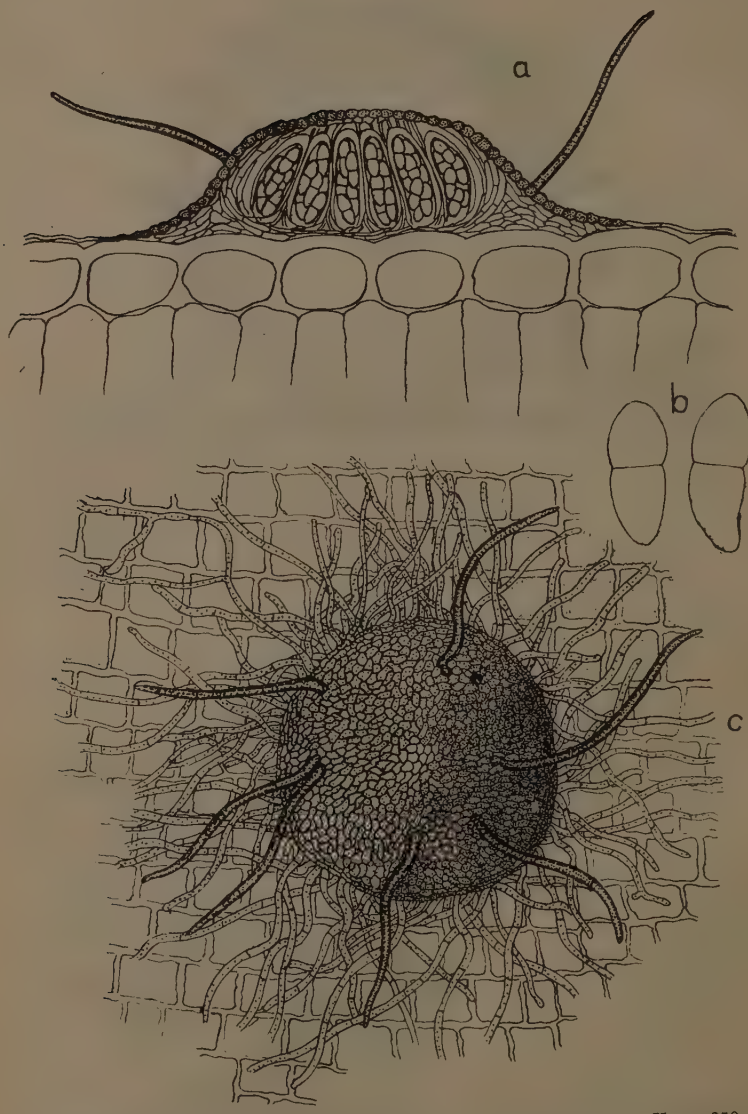

Abbildung 2. *Chaetothyrina Negii* (a) Schnitt durch einen Fruchtkörper Vergr. 250 x.; (b) Ascosporen Vergr. 1000 x; (c) Fruchtkörperansicht von oben Vergr. 250 x.

Plate II. *Chaetothyrina Negii* (a) Section through a fruit body Magnification 250 x; (b) Ascospores Magnification 1000 x; (c) Fruit body viewed from top Magnificacation 250 x.

Asci late ellipsoidei vel saccati, 32–42 x 12–14 μ, bitunicati, paraphysoides cellulatis circumdati, 8-spori. Sporae hyalinae, 12–14 x 5–6 μ, ellipsoideae, medium septatae et constrictae.

Hab. in foliis vivis *Taxi baccatae* L. subsp. *Walichianii* (Zucc.) Pilg. - India, Himalaya, Kumaon, Garhwal, Wan Valley, Wan. 31.5.1957.

Der Pilz wächst mit einem vollständig oberflächlichen, schmutzig olivenbraun gefärbten, aus 3–4 μ dicken Hyphen bestehenden Myzel auf den lebenden Nadeln des Wirtes. Die Myzelhyphen sind stark gekrümmt, septiert und ziemlich reich verzweigt und breiten sich locker über weite Strecken aus. Die ziemlich weit auseinanderstehenden, im Umriss rundlichen Fruchtkörper sind 100–120 μ im Durchmesser und 45–55 μ hoch, aussen mit wenigen, bis 80 μ langen, an der Basis bis 5 μ dicken, sich gegen das Ende stark verjüngenden, septierten, opak dunkelbraun en Borsten besetzt. Am Scheitel öffnen sich die Fruchtkörper mit einer flachen, unregelmässigen Mündung. Die Fruchtkörperwand ist zweischichtig; aussen befindet sich eine Lage von mäandrisch verschlungenen, 4–6 μ grossen schmutzig-olivenbraunen Zellen, die sich seitlich in das Myzel auflöst; innen besteht eine Schicht aus hyalinen, zum Teil deutlich zusammengepressten zartwandigen Zellen, welche auch an der Basis der Fruchtkörper deutlich zu sehen sind.

Die breit ellipsoidischen oder undeutlich sackförmigen bitunicaten, 32–42 x 12–14μ grossen, von zellig-faserigen Paraphysoiden umgebenen, 8-sporigen Asci neigen gegen die Mündung zusammen. Die Ascosporen sind ellipsoidisch, in der Mitte septiert und eingeschnürt, hyalin, 12–14 x 5–6 μ gross. (Abbildung 2.)

Die Gattung *Chaetothyrina* Theiss. basiert auf *Chaetothyrina musarum* (Speg.) Theiss; sie wird als *Chaetothyrium* mit zweizelligen hyalinen Sporen aufgefasst. Leider ist es schwierig zu entscheiden, wie weit die beiden Gattungen *Ceratochaeta* Syd. und *Microcallis* von *Chaetothyrina* Theiss. unterschieden werden können, doch ist der Name *Chaetothyrina* von diesen dreien der älteste und unser oben beschriebener Pilz entspricht ihr gut, wie eine Untersuchung der Typusart ergeben hat.

Summary

During an excursion, undertaken by the senior author under a Swiss Foreign Fellowship Programme, to the eastern Garhwal region of the central Himalaya, the authors found trees of *Taxus baccata* subsp. *Wallichiana* (Zucc.) Pilger, to be affected by a disease caused by a member of the Meliolaceae, in the valley of Wan. The fungus has been indentified as *Asteridiella taxi* (Sawada) Hansf. Another fungus was also found on the surface of the living leaves of the trees along with *A. taxi*. This fungus has been identified as *Chaetothyrina Negii* Müller and Bose sp. nov.

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STIMULATING EFFECT OF IONIZING RADIATION ON CERTAIN MICROORGANISMS

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Some reports of stimulating effect of radiation, especially ultra-violet rays, on microorganisms and higher plants have appeared earlier. Ramsey and Bailey (1930) showed marked increase in the spore production of ultra-violet irradiated cultures of *Alternaria tomato* (Cke.) Jones & *Fusarium cepae* Hanz emend Link & Bailey. In his later work Bailey (1932) observed that ultra-violet rays considerably hastened the initiation of sporulation in *F. culmorum*. Stimulation in the intensity of mycelial growth, stroma formation and colour of spore masses was also observed in many other Fusaria. When spores of *Penicillium chrysogenum* were subjected to very low dosages of ultra-violet rays, a greater number of visible colonies was formed in the treated series than in the control (Stauffer and Churchill, 1949). Stimulation due to mild intensities of radiation has also been reported for some specific physiologic processes in fungi such as citric acid formation in *Aspergillus niger* which was increased two to three folds (Kresling and Stern, 1936; Foster, 1949). Working with *P. notatum*, Jahiel *et al.* (1944) demonstrated the possibility of accelerating the growth of the fungus and decreasing the time of production of active penicillin *in vitro*, through radon gas and its decay products. Recently, Sokurova (1957) has reported stimulation in the growth of *Azotobacter chroococcum*, when exposed to low dosages of β radiation from various decay products of uranium.

Several workers have observed stimulation due to radiation in the rate of germination of seeds and yield of certain higher plants also. Kuzin (1956) in his elaborate studies has demonstrated the possibility of increasing the yield of certain crops with the help of ionizing radiation.

In the present investigation evidence has been obtained with regard to stimulation of biological activities of some microorganisms due to ionizing radiation. Fast neutrons, gamma and X-rays were employed in this study. Cultures of *Colletotrichum falcatum* Went (isolate No. 244) and *Ustilago nuda* (Pers.) Rostr. (G.C. No. 463) of the Indian Type Culture Collection, the causal organisms of red rot of sugarcane and loose smut of wheat, respectively, were used for this purpose. Heavy conidial suspensions from 14-day-old cultures of *C. falcatum* were prepared and washed twice with distilled water by repeated centrifugation. A mycelial suspension was prepared in case of *U. nuda* and washed similarly before irradiation. The method of exposure to fast neutrons was the same as reported by Bajaj *et al.* (1959). The spore suspension of *C. falcatum* and mycelial suspension of *U. nuda* were irradiated with fast neutrons at a flux of 0.35 x 10^7 ns./cm.^{2} /sec. for 10 minutes, 1, 2 and 4 hours and were stored at 7 °C. Irradiation with gamma rays and X-rays was

carried out at the Tata Memorial Hospital, Bombay. For gamma rays, Radon gas, obtained from Radium and collected in glass capillaries, was used and the capillaries were sealed in 0.5 mm. thick platinum capsules and put in a cavity made in a thick paraffin block so as to eliminate alfa and beta particles. The samples were arranged at a distance of 6.5 cm. from the centre of the capsules. These were exposed for different durations to obtain the total dosages of 30 r, 150 r, 750 r and 3,750 r. For X-rays, 220 KV and 15mA machine was used and dosages of 2,400r, 4,800r, 12,000r, and 24,000r were obtained by varying the time of exposure. The spore and the mycelial suspension treated similarly except for their exposure to radiation were kept to serve as control.

The germination of irradiated spores was tested on 0.1 per cent yeast extract and distilled water by the usual slide method at 25°C. The observations were recorded after 18 hours of incubation. About 500 spores were examined from at least four different drops.

The effect of radiation on the growth of the two fungi under study was determined by recording their dry mycelial weights. These were grown on 15 ml. dextrose-asparagine-phosphate medium and potato-dextrose solution, respectively, for 10 days in case of former and 10-30 days in case of the latter at 25°C. The dry mycelial weights were recorded by filtering through Whatman filter paper No. 42 and drying the contents at 60°C for 2 days.

Effect of Fast Neutrons: Germination studies of *C. falcatum* conidia exposed to fast neutrons revealed that at the lowest level of irradiation (10 min. exposure) a marked increase in the rate of germination was obtained. However, higher dosages considerably suppressed the germination (Table I). These results were confirmed by repeating the germination tests of the irradiated and un-irradiated spores under similar conditions.

Table I. Effect of fast neutrons (0.35×10^7 ns./cm.^{2} /sec.) on germination of *C. falcatum*.

Treatment	Per cent germination
Unirradiated (control)	37.4
10 min. irradiation	77.4
1 hour ,, ...	21.1
2 hours ,, ...	2.1
4 hours ,, ...	1.3

Stimulation was also observed in the amount of growth of *C. falcatum* obtained from the spores irradiated with the lowest dosage of fast neutrons as compared to that obtained from the un-irradiated ones. The amount of growth (dry mycelial weight) was nearly two and a half times more at this level of irradiation than the control, but it showed a decline as the

time of irradiation was increased to 1 hour (Table II) even though it was more than that of the control.

TABLE II. Effect of fast neutron irradiation on the growth of *C. falcatum*.

Treatment	Average dry weight in mg.
Unirradiated (control)	22.3
10 min. irradiation	58.0
1 hour	33.6
2 hours	—
4 hours ,, ...	—

Since the experiment on the growth was conducted after 35 days of storage of irradiated spores in frigidaire, the viability of the spores, which was already considerably low in the last two treatments, was completely lost.

In experiments with *Ustilago nuda*, the viability of the mycelial suspension, which was irradiated with fast neutrons for different periods, was tested by inoculating the fungus on potato dextrose-agar. There was no trace of growth in the treatments irradiated for one hour and above whereas 10 minute-irradiated cultures showed comparatively more growth than the control. In another experiment quantitative data were obtained by recording the amount of growth in terms of dry mycelial weight (Table III). It is evident from the table that the amount of the mycelial growth after 10-30 days of incubation in 10-minute-irradiated cultures always exceeded that in the control.

TABLE III. Effect of fast neutron irradiation (0.35×10^7 ns./cm.^{2} /sec.) on the growth of *Ustilago nuda*.

Period of incubation	Mycelial dry Wt. in mg.	
	Un-irradiated inoculum	irradiated inoculum
10 days	16.8	29.7
20 days	25.6	42.6
30 days	18.7	39.0

EFFECT OF GAMMA AND X-RAYS. Results of germination studies made on the spores of *C. falcatum* irradiated with varying dosages of gamma and X-rays showed marked acceleration at the lowest exposure of gamma rays, i.e., 30 r, which was more than 2 times that of the control (Table IV). There was a decline in the rate of germination as the dosages of gamma rays were increased but the percentage germination remained higher than that of the control upto 750 r exposure. In case of X-rays

an exposure of 12,000 r also exerted a marked stimulation in the rate of germination of spores of *C. falcatum* but the higher exposure suppressed the germination.

TABLE IV. Effect of gamma and X-rays on the germination of *C. falcatum*.

Gamma rays		X-rays	
Dose in roentgens	Per cent spore germination	Dose in roentgens	Per cent spore germination
30	63.3	2400	37.1
150	39.4	4800	48.0
750	46.0	12000	66.8
3750	24.8	24000	26.8
Control	26.4	Control	26.4

The phenomenon of stimulation due to low dosages of radiation has been a controversial issue because of the conflicting results reported earlier. Several workers failed to obtain any marked stimulation when different species of plants were irradiated with ionizing radiation but there are reports, particularly with regard to microorganisms which show positive stimulating action of radiation. Kuzin (1956), while discussing the phenomenon of stimulation, stated that the contradictory results obtained by different workers were probably due to imperfect techniques and differences in conditions under which the experiments were conducted. Our experiments clearly demonstrate the stimulating effect of radiation in low dosages so far as the germination of spores and the growth of certain microorganisms are concerned. The germination of irradiated spores of *C. falcatum* presents an interesting picture in that the exposure of 10 minutes to a neutron flux of 0.35×10^7 ns./cm.^{2} /sec. has accelerated it to nearly two times whereas the higher exposures of 1, 2 and 4 hours with the same flux have considerably suppressed it. Similar observations were made when the spores were irradiated with X-rays and gamma rays, though dosages required for such stimulation in the two cases varied considerably. It is difficult to explain as to how fast neutrons, gamma and X-rays in low dosages have accelerated or hastened the germination of conidia of *C. falcatum*. Sansome *et al.* (1945) have made similar observations in case of *Neurospora crassa* using X-rays and ultra-violet rays and have, however, suggested that acceleration of germination may be due to some action on spore membrane or cytoplasm that facilitates intake of water and thus hastens the germination. Stimulation in the germination of spores of *Tilletia foetida* when irradiated with X-rays and ultra-violet rays was also noticed by Berend (1954) and in case of Ultra-violet-irradiated sclerotia of *Sclerotinia sclerotiorum* by Bedi (1958).

The growth of *C. falcatum* and *U. nuda* obtained through the irradiated spores and mycelial bits, respectively showed positive trend of stimula-

tion due to low dosages of radiation. This is particularly interesting in case of *U. nuda* where mycelial bits were irradiated and these on subculturing yielded heavier growth.

Summary

A marked acceleration of germination and growth of conidia of *Colletotrichum falcatum* when irradiated with fast neutrons at a flux of 0.35×10^7 ns./cm.^{2} /sec. for 10 minutes was observed. Higher dosages, however, considerably suppressed the germination. Stimulation in germination was also noticed when spores were irradiated with 30 r of gamma rays and 12,000 r of X-rays. Similarly, mycelial suspension of *Ustilago nuda*, irradiated with fast neutrons, showed increase in the amount of growth.

Acknowledgment. The authors are grateful to Dr. H. J. Bhabha, and Dr. R. Ramanna, Atomic Energy Establishment, and the Superintendent, Tata Memorial Hospital, Bombay for providing necessary facilities of irradiation with fast neutrons and gamma and X-rays, respectively.

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SOME OBSERVATIONS ON UROMYCES INDIGOFERAE DIET. & HOLW., THE RUST OF INDIGOFERA LINIFOLIA RETZ.

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Introduction. One of the common diseases which affects *Indigofera* spp. is the rust caused by *Uromyces indigoferae* Diet. and Holw. (Syn. *U. orientalis* Syd.). Severe outbreaks of the rust, have been observed on *I. linifolia* year after year in Delhi and many parts of the country. Even though the rust has been reported long ago on *I. linifolia* by Sydow and Butler (1907), and also on *I. cordifolia*, and *I. glandulosa*, by Sydow and Butler (1912), Uppal, Patel and Kamat (1934), no work on its mode of perpetuation appears to have been done. Investigations were, therefore, taken up on certain aspects of the life cycle of the fungus, host range, physiologic specialization etc. and rust collections were made on *Indigofera linifolia* from Delhi, Bhopal and Saugar.

Field Observations. In nature, the rust on *I. linifolia* can be found all the year round. It has been observed under Delhi conditions that the uredial stage is quite abundant during the months of March-April, but with the rise in temperature, the teleutostage makes its appearance so that in the subsequent months both the stages can be found on the same plant. Under natural conditions, the uredospores of the pathogen infect the plant and the rust rapidly spreads, if conditions are congenial. Under adverse climatic conditions, such as high temperature of summer months or low temperature of winter months, the infection is not so abundant. It has also been observed that with the onset of monsoon, the infection, spreads rapidly, obviously due to the prevalence of more congenial conditions for the development of the pathogen.

Symptoms The uredosori and teleutosori are found on almost all the aerial parts of the plants but mainly are restricted to stem and leaves. They are amphigenous, scattered and round, 0.5 to 1.5 mm. in diameter. On stem, however, the uredosori are often oblong, minute, 0.25–0.75 mm. long, surrounded by ruptured epidermis with powdery mass of cinnamon colour.

Uredospores are globose, subglobose to ellipsoid, finely echinulate, brown with 3 equitorial germ-pores, measuring 18–23 μ x 14.6–22.0 μ.

Teleutospores are globose, subglobose, ovate or ellipsoid, apex round, strongly thickened (3.6–9.1μ) smooth, chestnut brown to brown in colour, 22–33 μ x 18–26 μ, epispore broad, (1.8–3.6μ), pedicel presistent, thick (3.6–5.5μ), hyaline or slightly coloured, yellow, upto 120 μ long.

THE UREDO STAGE: The minimum incubation period was 7 days at 28–37 °C. and 26 days at 10–12 °C. The uredospores germinated within the temperature range of 8–35 °C with optimum at 18–25 °C. Temperatures below 10 °C or above 35 °C appeared to be most unsuitable for uredospore germination. At 8–10 °C the germination was slow and germ-tubes of uredospores were either coiled, stunted or distorted, but on transfer to 25 °C the germination improved and the germ-tubes were normal. On the other hand, uredospores, kept for germination at 18–25 °C germinated readily producing normal germ tubes. The spores started germinating in less than $3\frac{1}{2}$ hours in congenial temperatures producing, in about 4 hours, germ tubes measuring 18μ to 58μ. The germ tubes elongated gradually and finally attained a length of about 840μ at the end of 24 hours. Material stored at 8–10 °C retained its viability for about 18 weeks (126 days). With the increase in the storage temperature the viability of the spores gradually declined. Thus at 40 °C the spores were viable for only about a week and at 60 °C they lost their viability within 48 hours. Material exposed to natural conditions at a temperature range of 4–44 °C with an average of 8° and 33 °C during experimental period, retained viability for nearly 16 weeks. Besides these, culture of the rust was maintained in the open and the viability of the uredospores was tested from time to time. The culture was maintained since April, 1956 and viability of uredospores was regularly tested at fortnightly intervals till September, 1957, and percentage of germination was found to vary from 80–95. The presence of uredospores throughout the year and the successful maintenance of the rust cultures in experimental plots, without resorting to further inoculation, sufficiently prove that the rust can perpetuate from year to year through the uredospores.

THE TELEUTO STAGE. Preliminary experiments on teleutospore germination of *U. indigoferae* (Syn. *U. orientalis*) indicated that even the fresh material germinated readily in a drop of water thereby showing that they do not have a dormant period. The spores started germinating after 48 hours and the percentage of germination gradually increased till the 6th day. In a typical experiment where fresh spores were kept for germination at 18–23 °C, the germination was 2%, 40%, 75%, 84%, 88% and 88% at the end of 48, 72, 96, 120, 144 and 168 hours respectively. There was a steep rise in the percentage of germination between 48-96 hours after which the rise was not much. To maintain the uniformity in taking down observations it was, therefore, considered proper to record observations 96 hours after setting up of the experiment.

The teleutospores germinated by producing a long hyaline promycelium measuing 28μ–38μ x 5.5–10.9μ with four septa, each cell containing a single nucleus. The sporidia and sterigmata were also hyaline, four in number measuring 3.5–14.5 μ x 3.5–11.0 μ, and 7.3–73.0 μ x 1.8–7.3 μ respectively.

The minimum, optimum and maximum temperatures for the germination of teleutospores were 15 °C, 18–20 °C and 35–37 °C respectively.

The material, collected in April, 1956, was stored in a frigidaire upto September 1956. In October that year the viability of the material was

tested and was found to be nearly 70%. This material was stored at 8–10°C, 25–26 C., 30°C. and 60°C. and also kept in a verandah and exposed to natural conditions. At 8–10°C teleutospores remained viable (above 5%) for nearly 15 months whereas those kept at 25°C and 30°C were viable even after 10 months. At higher temperature i.e. 60°C there was no deleterious effect for a week, after which, however, the spores were killed rather rapidly within next 8 days. Teleutospores exposed to natural conditions from October to July could successfully tide over adverse summer temperatures and remain viable (15% germination) at the beginning of the rainy season.

It therefore, appears that one of the mode of perpetuation of the rust could be through the teleutospores. Since the rust is not autoecious, as was proved by inoculation experiments on *Indigofera* spp. it is possible that the teleutospores might infect some other host to produce aecidial stage. The alternate host, however, has not yet been discovered.

Host Range and Physiologic Specialization of the Rust: To test the host range the following species of *Indigofera* were inoculated with the uredospores of the rust from *I. linifolia: I. glandulosa* Willd., *I. cordifolia* Heyne., *I. enneaphylla* Linn., *I. arrecta.*, *I. hirsuta* Linn., *I. tinctoria* Linn., *I. tinctoria* var. *sumatrana* Gaertn., *I. anil* Linn., and *I. linifolia* Retz. (control).

Only *I. linifolia* got infected. It is, however, very interesting to note that the plants of *I. glandulosa* and *I. cordifolia* could not be got infected even though occurrence of *U. orientalis* (Syn. *U. indigoferae*) has been recorded on them. Due to the non-availability of the rust cultures from these hosts, back inoculations were not possible and hence no definite conclusions can be drawn at this stage.

The host range studies, as mentioned above, gave the first indication that rust samples under study, though morphologically alike, may be pathologically different. The existence of such forms in rusts is well known.

To study the physiologic specialization in *U. indigoferae*, the two samples. one from *I. tinctoria*, collected from Bapatla, (Andhra-Pradesh) (Joshi and Reddy-1958), and other from *I. linifolia* were taken. Plants of *I. tinctoria*, *I. linifolia* *I. cordifolia*. and *I. glandulosa* were raised under identical conditions and inoculated in two lots with the uredospores of the rusts from *I. tinctoria* and *I. linifolia* separately. It was found that the rust from *I. linifolia* did not infect *I. tinctoria* and *vice versa* and hence they could be treated as two distinct forms of *Uromyces indigoferae* on the basis of their pathological characters. Since there is no morphological differentiation between the two samples it is proposed to name them as *U. indigoferae* f. sp. *linifoliae* and *U. indigoferae* f. sp *tinctoriae*.

Summary

The uredospores of the rust germinate readily within less than 3½ hours at temperature range of 8–35°C., the optimum temperature being

18–25°C., and they are chiefly responsible for the perpetuation of the disease from season to season. The teleutospores of the rust do not require a dormant period. The optimum, minimum and maximum temperature for their germination being 18°–20°C., 15°C and 35°–37°C respectively. Teleutospores are quite hardy and can successfully tide-over summer months. It is, therefore, possible that one of the sources of perpetuation of the rust may be through the teleutospores. Since the rust is not autoecious the possibility of the existence of an alternate host cannot be ruled out.

The rust *U. indigoferae* from *I. linifolia* cannot infect *I. tinctoria* and *vice versa* and hence they are two distinct forms of *Uromyces indigoferae*, one restricted to *I. linifolia* and the other to *I. tinctoria* and it is proposed to name them as *Uromyces indigoferae* f. sp. *linifoliae* and *Uromyces indigoferae* f. sp. *tinctoriae*.

ACKNOWLEDGMENT. Our grateful thanks are due to Dr. R. S. Vasudeva, Head of Division of Mycology and Plant Pathology, for suggesting the problem and for his guidance during the course of investigation. Thanks are due to Dr. R. Prasada, Mycologist, for his helpful suggestions and for going through the manuscript. Help rendered by Shri K. R. Sreekantiah and Miss D. Kak during the course of these studies is also thankfully acknowledged.

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ON A NEW PARASITIC FUNGUS ON CASTANOPSIS.

Emil Müller and B. D. Sanwal

(Accepted for publication April 10, 1959)

The genus *Microstroma* Niessl, with the type species *Microstroma album* (Desm.) Sacc. (described as *Microstroma quercigenum* by v. Niessl, 1861) was originally considered to be a hyphomycetous fungus. By placing this fungus in the family Exobasidiaceae (Basidiomycetes) because of the constant number of spores on the "basidium", Schroeter (1888) initiated an argument which uptil now has not been clarified. Many authors like Hennings (1900) supported Schroeter while others like Niessl and Lindau (1901) still considered it to be an imperfect fungus.

The first experiments to clear this confusion were done by Maire (1906) and he reached the conclusion that the spores could in no way be considered as basidiospores. More recently, Wolf (1927, 1929) who investigated this question again could not prove that the genus belonged to the Basidiomycetes. As a result of cytological work on *Microstroma juglandis* (Ber.) Sacc. (not the type species), Pires (1928) however, considered the fungus to belong to the Basidiomycetes. Von Arx (1957) placed the genus *Microstroma* between *Kabatiella* Bub. and *Aureobasidium* Viala et Boy., thus indicating close relationship of the above genera.

There are not only different opinions regarding the placement of the genus but also with regard to its morphology. Patouillard (1902) observed in material of north African origin that the conidiophores arise not in one layer but come out of the stroma from different points. Because of this character, a new genus, *Helostroma*, was erected by him which was different from *Microstroma* in this characteristic.

Maire (1913) investigated the fungus again and confirmed the findings of Patouillard (1902) in toto, We would therefore consider the morphological descriptions of *Microstroma album*, the fungus discussed below and that given by Patouillard (1902) in a diagram as correct.

Unfortunately, Maire (1913) has not discussed the nomenclatorial consequences arising from his work. Patouillard (1902) seems to be of the opinion that Niessl's *Microstroma quercigenum* (Opitz) Niessl and his own *Helostroma album* (Desm.) Pat. may be different. According to Maire (1913) this is not the case and *Microstroma* Niessl and *Helostroma* Pat. have the same type species and are, therefore, synonymous, whereby *Microstroma* Niessl gets without doubt the priority due to its being the older name.

Together with Mr. S. K. Bose, Ranikhet (Almora, U.P.), we found in the Western Himalayas a parasite on living leaves of *Castanopsis tribuloides* A. DC. which is certainly closely related to *Microstroma album*

but is different in some ways to warrant its inclusion in separate species. The fungus produces Witches'-broome, where all the leaves arising on one branch show fructifications of the parasite on their surfaces. The arboreal growth of the conidiophores also seem to be more clear in this fungus. Each conidiophore carries 8 conidia in contrast to *Microstroma album* where most of the authors agree that there are only six.

Because we consider *Microstroma* and *Helostroma* as synonymous, we name this fungus *Microstroma castanopsis* nov. spec. The description follows:

Microstroma castanopsis nov. spec.

Mycelium in foliis vivis parasiticum, maculae irregulariter rotundatae, clariorae formatum, stromatis globsis hyalinis, 30–35μ diam. aggregatum et loculi stomatici completum. Hyphae sporophorae numerosae, dense fasciculatae et columne formantae, per stomata saepius exeuntia. Columna, superficialia albida, ramosa et arbor pyramidatus formantus. Conidiophorae solitariae curvatae, in extremis clavatae, 8 conidiis fusoidis hyalinis, 6–8 x 1, 5–2μ congestis.

Hab. in foliis vivis *Castanopsis tribuloidis* A. DC. - India, Kumaon, Bindsar (Almora) 23.5.1957.

The fungus causes bushy Witches'-broome to develop on the affected trees, out of which a large number develop further into shorter branches. The leaves produced on these branches are without exception diseased. The fungus produces large or small spots which sometimes cover the whole leaf surface. The spots are irregular, sharply defined from healthy areas, on the upper surface light green and on the lower surface brown coloured.

The fructification break through the lower surface. In the stomatal cavities, (mostly continuous stomata), a compact, hyaline, rounded stroma, measuring about 30–35μ in diameter is produced. On the outside the stroma is surrounded by a layer of concentric, hyaline, more or less compressed, 4–7μ long, thin walled cells. Inside, right from the base, there arise rows of hyaline cells which are more or less prismatic and thin-walled. These accumulate near the stomatal aperture and elongate to the outside. From the stroma hyphal complexes penetrate the leaf tissue. At first they produce irregular, bulbous stromatic bodies, the cells of which separate from each other and grow into cellular hyphae between the host cells of the whole tissue.

The bundle of stroma cells present near the stomatal aperture penetrate to the outside, widen themselves above the opening into ill defined club like structures and envelop like a collar a bundle of diverging conidiophores which are also club-shaped. Inside this second crown, a third arises similarly consisting of conidiophores and further inside a fourth and sometimes a fifth, whereby the number of conidiophores decreases from below to upwards. As a whole this fructification looks like a 30–40μ high pyramid shaped, richly branched tree which shows in the lowest

optical section 6–9 conidiophores and in the uppermost only 1–3. These are, as much as one can make out, 7–10μ long, bent on the outside and approximately 4μ wide. The unicellular hyaline spores measuring 6–8μ in length and 1. 5–2μ in breadth are spindel-shaped and cut off from short stalks. The number of spores per conidiophore is eight.

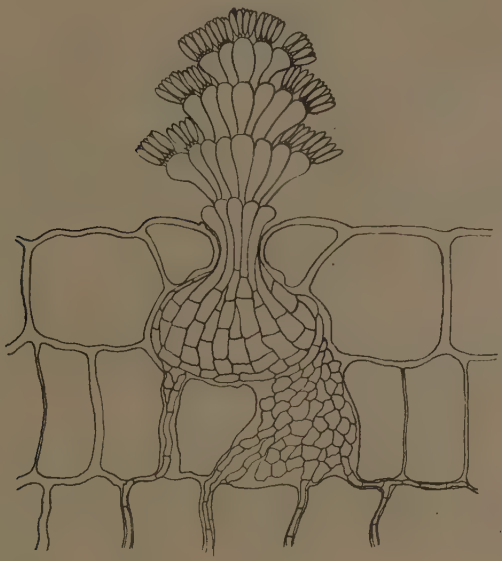

Figure 1. Section through a part of a leaf infected by *Microstroma castanopsis*. 750 x

In the same way as *Microstroma album* (Desm.) Sacc. one is in doubt whether the fungus described above can be considered as an imperfect or a Basidiomycete. The biological similarity to Exobasidiaceae is increased by the fact that this fungus has the capacity to produce Witches'-broome, Morphological investigations only cannot give a solution to the controversial question; some cytological investigation should be done to arrive at a definite solution, but unfortunately we have only dead material in our hands.

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THE MYXOMYCETES OF THE MUSSOORIE HILLS XII

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(Accepted for publication March 15, 1959)

The first eleven contributions (listed under references) describe 70 known species, 5 new species and 1 new form. This twelfth paper on the series deals with 8 more species of which 7 are new records from the Mussoorie Hills while 4 are new records for India.

The classification of Martin, 1949, has been followed throughout this study although monographs of Lister and Lister, 1925, and Macbride and Martin, 1934, were freely consulted.

The number of the species are the serial numbers of the myxomycetous flora of the Mussoorie Hills.

The collections have been deposited in the Herbarium of the Punjab University and Herbarium Crypt. Ind. Orient. New Delhi. Duplicate material is also deposited in the Herbarium of the State University of Iowa, U.S.A.

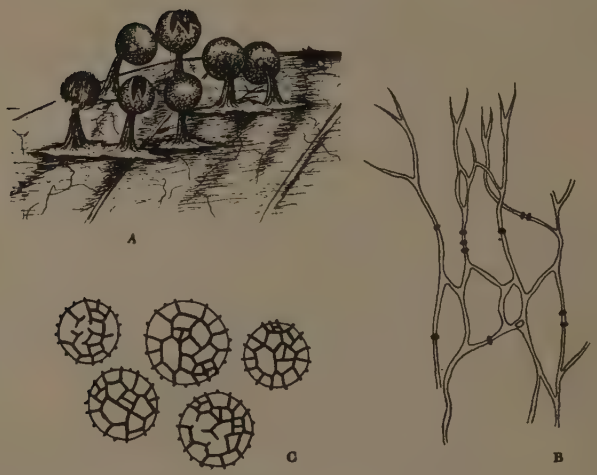
77. *Diachea subsessilis* Peck.

Fructifications sporangiate; sporangia gregarious, stipitate, iridescent, dove-coloured or like a pigeon's neck due to iridescence, purplish with bluish reflection below and dark gray or brass-coloured above, globose, rough or uneven, 0.37–0.5 mm. in diameter; stipe erect, snow white, calcareous, usually smooth, sometimes slightly rough, brittle, gradually tapering above, 0.75–1 mm. long and up to 0.12 mm. wide at the base; hypothallus extensive, white, venulose, forming a network supporting the snowy stipes, calcareous; peridium, single, thin, membranous, iridescent, rough, noncalcareous; dehiscence, irregular, the peridium rupturing at the top while its lower portion usually remains intact.

Columella distinct, minute, simply a prolongation of the stipe, snow-white, conical, calcareous, up to 65 μ long.

Capillitium well developed, arising from the entire length of the columella, composed of delicate threads free from lime; capillitial threads violaceous brown, branched and anastomosed to form a lax network, ultimate branchlets free, attenuated and hyaline, threads possessing darker, complete rings of thickenings at places.

Spores black in mass, dark violet under the microscope, globose to subglobose, reticulate-verrucose, reticulations mostly complete and irregular, sometimes incomplete, warts small and few, 8.8–11.2 μ in diameter.



Text—Fig. 1. *Diachea subsessilis* Peck,
A. Fructifications, X 20. B. Capillitial threads marked by rings of thickening, X 320.
C. Strongly reticulate-verrucose spores, X 1150.

Collected on dead leaves and dead twigs of various deciduous trees and on green herbs, Nala Pani, Dehra Dun, Aug. 7, 1954, **322**. New record in India.

This Mussoorie collection undoubtedly belongs to *Diachea subsessilis* Peck and its strongly reticulate spores are quite diagnostic of this species. Its stipes are long for *D. subsessilis* but according to Martin (personal correspondence, June 2, 1958) there are some specimens with stipes nearly as long.

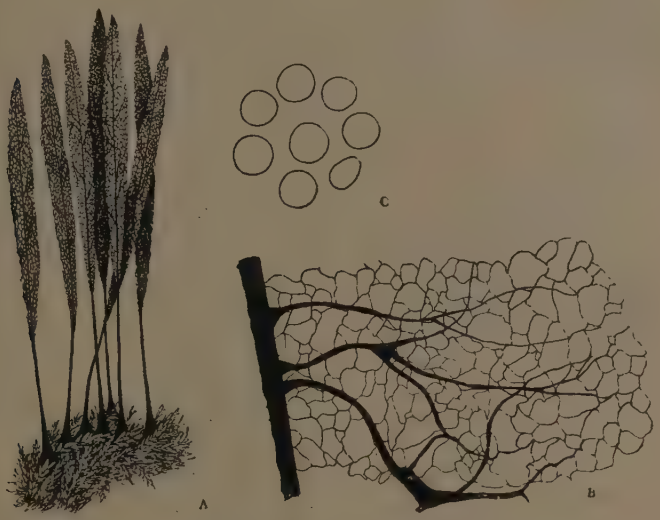
78. *Stemonitis axifera* (Bull.) Macbr.

Fructifications sporangiate, total height up to 13 mm.; sporangia gregarious, occurring in small but dense clusters of up to 50 sporangia per cluster, stipitate, erect, ferruginous, cylindrical, narrower below, then broader upwards and finally again narrowing down with tips tapered, more or less accuminate, 4.5–6.5 mm. long and up to 0.45 mm. wide; stipe erect, black and shining, rigid, solid, smooth, gradually tapering upwards, swollen at the base, 4–7 mm. long and up to 0.15 mm. wide at the base; hypothallus common to a patch, dark brown to black; peridium not observed; dehiscence irregular.

Columella prominent, central, black, simply a prolongation of the stipe, gradually tapering upward, dissipating before reaching the tip of the sporangium, gives out capillitium all around.

Capillitium prominent, brown, primary branches thick, large, dividing, anastomosing and tapering, dilated at their axils, capillitial branches united at the surface to form a lighter coloured peridial net with fine meshes, meshes up to 45 μ in diameter.

Spores dark brown in mass, pale brown under the microscope, globose, subglobose or ellipsoid, smooth, 4.8–6 μ in diameter, 4.8–6.4 x 4–5.6 μ when ellipsoid.



Text—Fig. 2. *Stemonitis axifera* (Bull.) Macbr.
A. Sporangial cluster, X 5. B. Part of columella and capillitium, X 320. C. Spores, X 1150.

Collected on dead wood of a tree and on living green mosses, The Park, Mussoorie, July 23, 1954, 323. New record in India.

The species is marked by long, ferruginous sporangia, close-meshed surface net of the capillitium and smooth walled, spores, 4.8–6 μ in diameter.

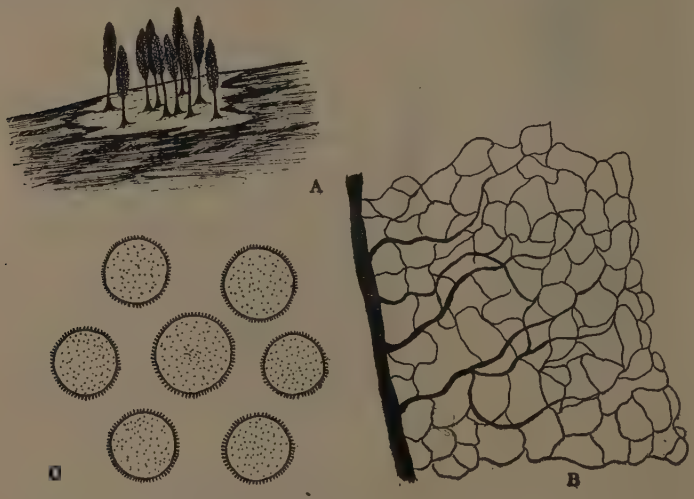
79. *Stemonitis pallida* Wingate

Fructifications sporangiate, total height up to 2 mm; sporangia gregarious, stipitate, erect, dark brown, cylindrical, narrowed at the base, with rounded apex, 1–1.25 mm. long and up to 0.38 mm. wide; stipe erect, black, shining, gradually tapering upward, smooth, rigid, solid, 0.3–0.6 mm. long and up to 75 μ wide at the base; hypothallus common to a patch, shining, silvery; peridium not observed; dehiscence irregular.

Columella percurrent, ending abruptly just below the apex, central, simply a prolongation of the stipe, black, giving out capillitial branches for entire length.

Capillitium abundant, dark violaceous brown, primary branches thick, large, branching and anastomosing, of uniform thickness, for the most part not dilated at their axils; capillitial branches united at the surface to form a close-meshed peridial net which is, however, incomplete or lacking at the top, meshes mostly small, irregular in shape and size, mostly up to 45 μ in diameter, rarely elongated up to 60 μ but the width remaining below 45 μ, ultimate capillitial branches free at the top of the sporangia and mostly swollen or knob-like at their apices.

Spores, dark-brown in mass, light brown to brown under the microscope, globose to subglobose, minutely and profusely verrucose, 6.4–8 μ in diameter.



Text—Fig. 3. *Stemonitis pallida* Wingate
A. Sporangia, X 5. B. Capillitium, X 320. C. Spores, X 1150.

Collected on dead wood, Saharanpur Road, Dehra Dun, August 6, 1954, 324.

This Mussoorie collection closely resembles *Stemonitis pallida* Wingate except that its sporangia are somewhat small for the species.

80. *Stemonitis herbatica* Peck

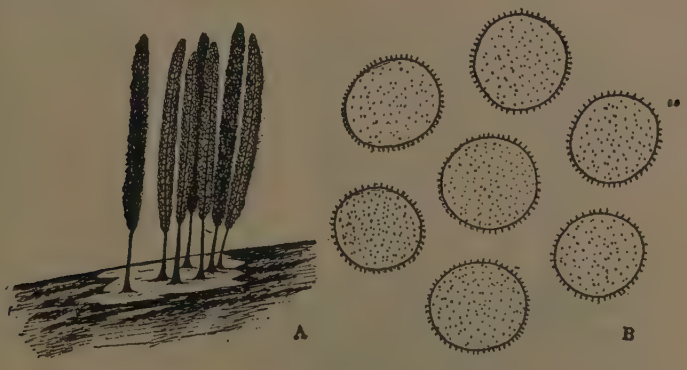
Fructifications sporangiate, total height up to 6 mm.; sporangia gregarious, in small to large clusters; clusters very close to each other

with 9–35 sporangia in a cluster, the occurrence of sporangia in clusters being a very characteristic feature and giving bushy appearance to the plants; sporangia closely clustered but separate from each other, dark brown, cylindrical, bent at the apex, apex obtuse, 4–4.5 mm. long and 0.25–0.3 mm. wide; stipe slightly expanded at the base, black 0.38–1.2 mm. long and up to 0.07 mm. wide at the base; hypothallus conspicuous, dark red, shining, membranous, common to a patch; dehiscence irregular.

Columella prominent, black, central, unbranched, simply a prolongation of the stipe, tapering towards the apex, ending just below apex and becoming very fine and wavy, giving out capillitium on all sides of columella throughout its entire length.

Capillitium consisting of violaceous threads which arise from the columella and are repeatedly branched and anastomosed to form a net, the threads thickened and with plate-like expansion at the point of origin from the columella and other branches near the columella. No such thickenings are observed at the periphery. Therefore, a net near the columella is coarser and is composed of thick threads and wider meshes, while towards the periphery the net is very fine, i.e. consists of fine threads and very fine meshes; 4–18 μ in diameter. The net is closed and without any free ends.

Spores dark brown in mass and pale brownish under the microscope, minutely verrucose, wall prominently darker, globose to subglobose, 6.4–8μ in diameter.



Text—Fig. 4. *Stemonitis herbatica* Peck,
A. Fructifications, X 5. B. Profusely and distinctly verrucose spores, X 1150.

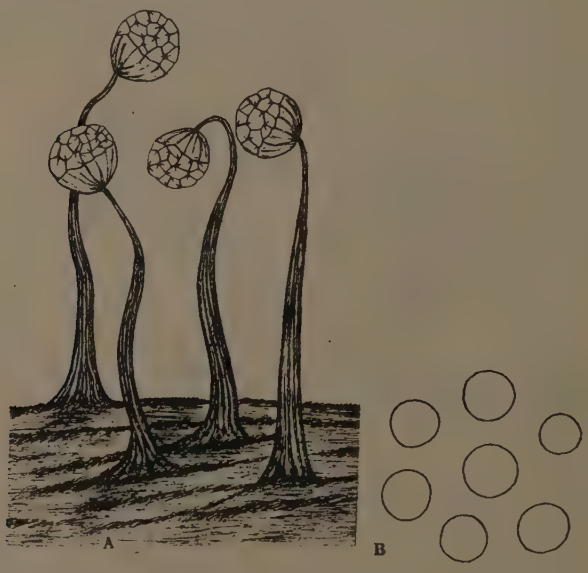
Collected on green herbaceous plants and dead and decaying wood, Doiwala, Dehra Dun, August 11, 1953, 325.

This Mussoorie collection undoubtedly belongs to *Stemonitis herbatica* Peck. It can be easily differentiated from *S. pallida* Wingate in possessing a complete surface net throughout.

81. *Cribraria languescens* Rex.

Fructifications sporangiate, total height up to 3 mm.; sporangia loosely gregarious, stipitate, erect or nodding, brown, globose, 0.45–0.55 mm. in diameter; stipe long, erect, or bent, flexuous, dark brown below and brown above, tapering upwards, longitudinally rugose, 2–2.5 mm. long and up to 0.37 mm. wide at the base; hypothallus small, membranous, black, rotate; peridium single, very thin and membranous, spreading in between the meshes of the net, iridescent, represented at the base by a cup-shaped calyculus; calyculus conspicuous, more than 1/3 of the sporangium, iridescent, thin, membranous with even margin, marked on the outside by minute or by more conspicuous granular ribs radiating out from the stipe, calyculus gives rise from its margin to elongate nodular or internodal projections or ribs which soon branch and anastomose above to form a brown network; nodes large, elongated, dark brown, filled up with brown granules, giving off several internodal and free branches; internodes brown, slender, single; free ends numerous and given out from the nodes; meshes of net large and irregular.

Spores brown in mass, pale brown under the microscope, globose, smooth, 4.8–6.4 μ in diameter.



Text—Fig. 5. *Cribraria languescens* Rex.
A. Sporangia, X 20. B. Spores, X 1150.

Collected on decaying wood of a deciduous tree, Chakrata Toll, Mussoorie, Sept. 10, 1954, **327**. New record in India.

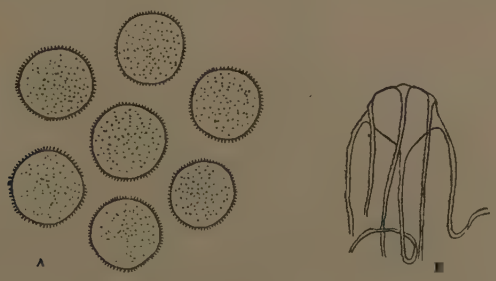
This Mussoorie collection belongs to *Cribraria languescens* Rex but differs from it in possessing somewhat smaller spores and more free ends in the net.

82. *Dictydiaethalium plumbeum* (Schum.) Rost.

Fructifications pseudoaethaliate; pseudoaethalia scattered, chestnut brown, or brown to dark brown, resupinate, irregular in outline, more or less circular, becoming cracked when old, extending up to 8.3 cm., up to 1.2 mm. thick, surface areolate, areolae hexagonal. These aerolae correspond to sporangia which are closely packed together into pseudoaethalium; sporangia cylindrical, darker at the top and fading lower down, 0.3–1.2mm. tall and 0.1–0.2 mm. wide, sporangial wall evanescent between the threads but persistent at the top forming a flat to dome shaped hexagonal cap, each of the six angles of the cap, giving out threads of the pseudocapillitium; threads 3–6 μ wide, running down to the base of sporangia, brown to light brown, branched, rarely anastomosed, usually roughened on one side due to spines or warts, sometimes roughened on both sides, also marked by faint transverse lines or trabeculae placed irregularly along the entire length; hypothallus shining, silvery, cream coloured, membranous, abundantly developed and surrounding the pseudoaethalium; dehiscence not observed. (Plate I)

Pseudocapillitium represented by the threads of the sporangia hanging down from the angles at the top of the sporangia and running to their bases as described above.

Spores brown, sometimes bright yellow in mass, subhyaline to pale yellow or pale brown under the microscope, globose to subglobose, uniguttate, gutta large, filling one-half to three fourth of the spore cavity, minutely to faintly verrucose, 9–12(–15)μ in diameter.



Text—Fig. 6. *Dictydiaethalium plumbeum* (Schum.) Rost.
A. Spores, X 1150. B. Pseudocapillitium, X 80.

Collected on decaying logs, Dehra Dun, August 20, 1953, **328**. On bark of dead trees, Doiwala, Dehra Dun, August 25, 1953, **329**. New record in India.

Both these Mussoorie collections belong to *Dictydiaethalium plumbeum* (Schum.) Rost. The yellow and somewhat larger spores (9–12μ) of n. 328 appear to be the result of very rapid and somewhat incomplete maturation. This is even more so in the case of n. 329 which shows spores up to 15 μ in diameter.

83. *Perichaena vermicularis* (Schw.) Rost.

Fructifications plasmodiocarpous; plasmodiocarps dull brown, concolorous with substratum and easily overlooked, typically like a plasmodium, forming complete reticulations, terete, arcuate or annular, 0.25–0.4 mm. in diameter; hypothallus well developed, membranous, dark brown; peridium single, thin, membranous, dull yellow or dark cincereous, minutely wrinkled; dehiscence irregular, the peridium rupturing at the top irregularly while the lower portion persists.

Columella absent.

Capillitium abundant, composed of yellowish brown, branched threads, the capillitial threads uniform in thickness, up to 3.2 μ thick, prominently and profusely verrucose.

Spores dull yellow in mass, pale yellow under the microscope, uniguttulate, guttule filling up about ½ or less of the spore, globose to subglobose, sometimes oval or elliptical, minutely but profusely verrucose, 11–14 μ in diameter.

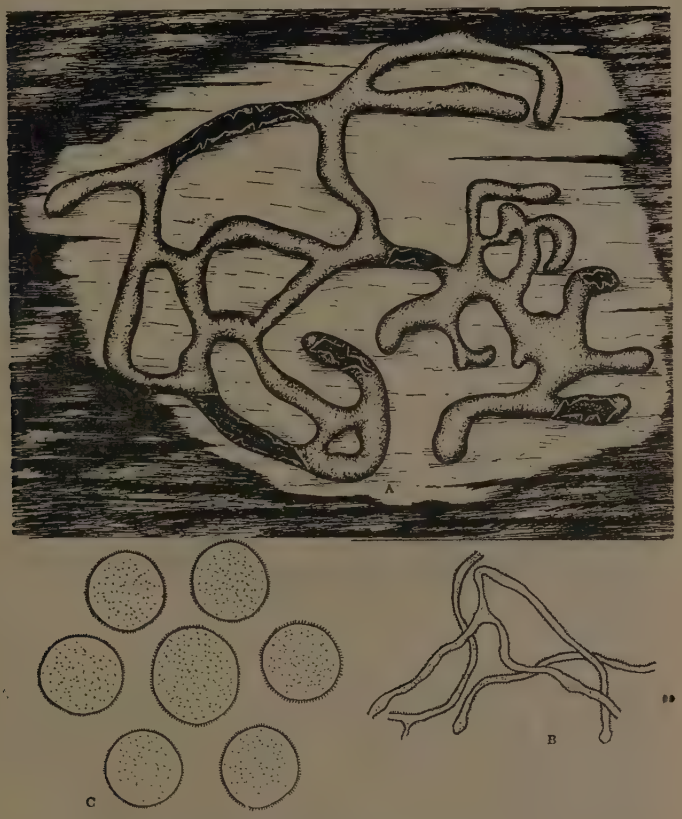
Collected on bark of a dead tree, Kansrao, Dehra Dun, August 13, 1953, **330**.

This Mussoorie fungus undoubtedly belongs to *Perichaena vermicularis* (Schw.) Rost. except that its peridium appears to lack outer granular layer.

84. *Perichaena depressa* Libert

Fructifications sporangiate, superficially appearing as an aethaliate mass; sporangia densely packed together in irregular and small to large patches, strongly depressed and the whole sporangial cluster appearing like a thin crust over the substratum, polygonal due to mutual compression, sessile, brown to chestnut brown, sometimes darker or dark brown to dark chestnut brown, 0.25–2.25 mm. in diameter; hypothallus absent; peridium single, thick, densely covered with dark brown granules, tough or cartilaginous, concolours on both sides, thin, membranous, shining or iridescent and lacking the dark brown granules on the periphery; dehiscence circumscissile, the thin membranous peripheral region of the peridium easily rupturing or becoming detached at the margin and thereafter the whole peridium at the top coming off as a lid. The lid when fully

detached is beautifully marked by thin, shining, membranous cortina on its periphery.



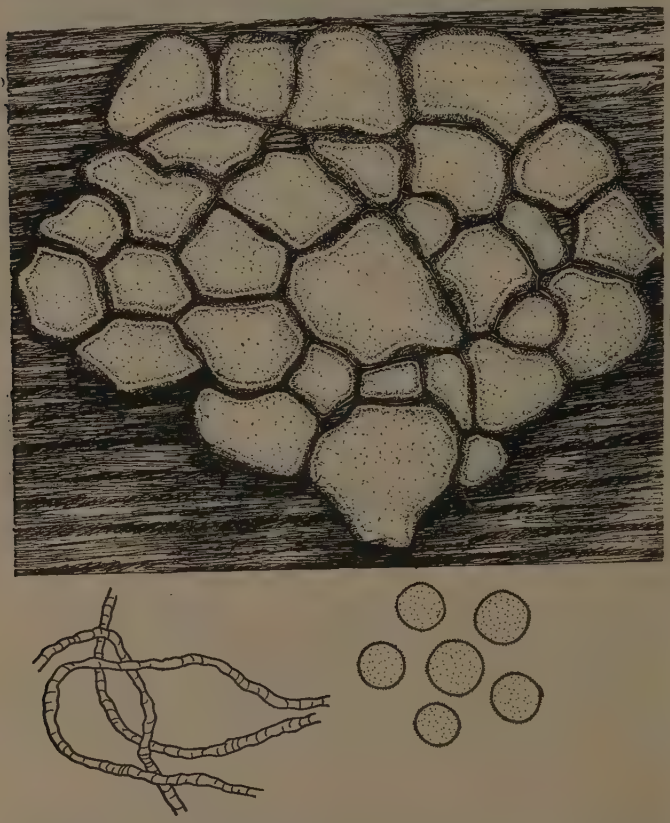
Text—Fig. 7. *Perichaena vermicularis* (Schw.) Rost.

A. Plasmodiocarps with copious hypothallus, X 20. B. Profusely verrucose capillitial threads X 320. C. Minutely verrucose spores, X 1150.

Columella absent.

Capillitium abundant, sometimes sparse to scanty and even lacking, composed of a tangled mass or net formed by long, sparingly branched yellow threads; threads intertwined, flexuous, looped, up to 3.2 μ wide, more or less of uniform width throughout, rough, marked by abundant usually closely placed, faint, incomplete cogs as in certain Arcyrias, but somewhat wider.

Spores yellow in mass, yellowish orange to orange with age, light yellow under the microscope, globose to subglobose, minutely and profusely verrucose, 8–11.2 μ in diameter.



Text—Fig. 8. *Perichaena depressa* Libert

A. Closely packed sporangia, X 20. B. Capillitial threads marked by incomplete cogs, X 320. C. Minutely verrucose spores, X 1150.

Collected on dead wood of a deciduous tree, Doiwala, Dehra Dun, August 9, 1954, **331**. On bark of a tree, Raiwala, Dehra Dun, August 15, 1954 **332**.

These two Mussoorie collections undoubtedly belong to *Perichaena depressa* Libert. The collection n. 332 closely resembles *P. quadrata* Macbride as described by Macbride and Martin, 1934. However, Martin, (1949), regards it as synonymous with *P. depressa*. According to him

(personal correspondence, June 19, 1958) *P. quadrata* is no more than a small form of *P. depressa*.

PLATE I

Dictydiaethalium plumbeum (Schum.) Rost., showing pseudoaethaliate fructification, cracked with age.

ACKNOWLEDGMENTS: The authors are deeply indebted to Dr. G. W, Martin of the State University of Iowa for help in the determination of the species and Prof. P. N. Mehra, Panjab University Botany Department, for providing facilities and encouragement. They are also thankful to Mr. B. Khanna for making illustrations of the fruit bodies.

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A MOSAIC DISEASE OF CHINESE SARSON (BRASSICA JUNCEA (LINNAEUS) COSS. VAR. RUGOSA ROXB.)

R. N. Azad and O. P. Sehgal

(Accepted for publication March 15, 1959)

Introduction. During the course of survey for plant virus diseases in Simla Hills, a severe mosaic disease was observed in Chinese *sarson* (*Brassica juncea* (Linnaeus) Coss. var. *rugosa* Roxb.) in November, 1953. This variety of leaf-mustard which is said to have been originally introduced from Tibet is grown as winter crop in the Himalayan region of India, Bhutan, Nepal etc.; its leaves are used, both green and after drying, for culinary purposes (Anon., 1948).

The results of investigation on host-range, symptomatology, properties and insect-transmission of the causal virus are presented in this paper.

Materials and Methods. The diseased material was collected from Simla area and a culture of the virus was established by sap-inoculation on *Brassica juncea* var. *rugosa* and *B. nigra* (L.) Koch plants on which it was maintained throughout the course of the investigation.

While making sap-inoculation carborundum powder was invariably used as an abrasive. For insect-transmission studies, virus-free colonies of *Brevicoryne brassicae* Linnaeus and *Myzus persicae* Sulzer were maintained on young plants of cabbage, and of *Aphis gossypii* Glover on chilli which were found immune to the virus.

All investigations were carried out in an insect-proof glass-house and the usual precautions were observed.

Symptoms of the Disease. The diseased Chinese *sarson* plants showed vein-clearing green vein-banding, mottling and severe puckering of the leaves (fig. 1). The affected plants were stunted and flowering was either absent or scanty, producing only a few poorly-filled and shrivelled fruits. In advanced stages of infection the stem and fruits also showed distinct mottling. The disease incidence was fairly high resulting in considerable loss in yield.

Experimental

Transmission. The virus was found to be readily transmissible by sap-inoculation and also by three species of aphids, viz., the melon aphid (*Aphis gossypii* Glover), the mealy cabbage aphid (*Brevicoryne brassicae* Linnaeus,) and the green peach aphid (*Myzus persicae* Sulzer). The virus was not seed-borne which was determined in case of *Brassica juncea* var. *rugosa* and *B. nigra* by making observations on a large number of seedlings raised from seeds collected from diseased plants.

Fig. 1. Symptoms on Chinese *Sarson* (*Brassica juncea* var. *rugosa*.)

Host-range and Symptomatology. The virus was successfully transmitted mechanically as well as by aphids to *Brassica juncea* (Linn.) Coss. var. *rugosa* Roxb., *B. nigra* (L.) Kock, *B. campestris L.* var. *sarson* Prain, *B. campestris* L. var. *toria* Duthie and Fuller, *B. hirta* Moench, *B. napus* L., *B. chinensis* L. (Chinese cabbage vars. *typica*, Chi-hi-li and Wong Bok), *B. pekinesis* (Lour.) Rupr. (Chinese cabbage var. Pe - Tsai), *Eruca sativa* Mill., *Erysimum* sp., *Hesperis matronalis* L., *Iberis* sp., *Lepidium sativum* L., and *Mathiola incana* L.

In addition, the virus was transmitted to *Brassica rapa* L. (turnip, var. Purple Top White) but only by the aphid vector, *Brevicoryne brassicae*, and attempts to infect this host by sap-inoculation remained unsuccessful.

The virus was also transmitted by sap-inoculation to *Zinnia elegans* Jacq. var. Giant of California and *Nicotiana tabacum* L. varieties White Burley and Harrison's Special. The transmission to *Z. elegans* and N. *tabacum* as also to cruciferous hosts was confirmed by making back inoculations on *Brassica nigra*. The symptoms produced on some of the important hosts are described below:—

(i) *Brassica nigra:* Vein-clearing followed by mottling, severe malformation of the young leaves and arresting of apical growth (fig. 2). The fruits in severely diseased plants were either poorly-filled or entirely lacking.

(ii) *Brassica campestris:* Young plants of *B. campestris* var. *toria* reacted with vein-clearing followed by blotchy mottle, slight puckering and persistent green vein-banding (fig. 3) . The symptoms on var. *sarson* were mild as compared to var. *toria*.

(iii) *Brassica chinensis:* After about 12–14 days of inoculation the new leaves of *B. chinensis* var. *typica* showed pronounced vien-clearing followed by mottling and slight vein-banding (fig. 4). The var. Wong

Fig. 2. Symptoms on *Brassica nigra*.

Fig. 3. Symptoms on *Brassica campestris* var. *toria*.

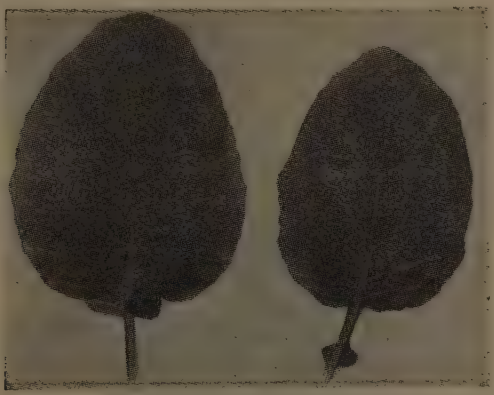

Fig. 4. Symptoms on *Brassica chinensis* var. *typica*.

Bok showed only a transient mild mottle along the veins whereas the var. Chi-hi-li carried the virus almost symptomlessly.

(iv) *Lepidium sativum:* The new leaves formed after about 14 days of inoculation, which was done on cotyledonary leaves, were reduced in size and the entire crown showed conspicuous stunting.

(v) *Mathiola incana:* The leaves showed diffused chlorotic areas followed by a characteristic 'breaking' of colour of the corolla.

(vi) *Zinnia elegans:* Conspicuous vein-clearing after about 10–12 days of inoculation followed by distinct light green diffused mottle (fig. 5). The petals of flowers also sometimes showed mottled appearance.

Fig. 5. Symptoms on *Zinnia elegans.*

(vii) *Nicotiana tabacum:* Only inoculated leaves showed symptoms consisting of local-lesions in the form of small necrotic pin-points surrounded by a chlorotic halo (fig. 6). Almost identical symptoms were observed on vars. White Burley and Harrison's Special. The virus could be recovered from leaves showing local-lesions on sub-inoculation to *B. nigra.*

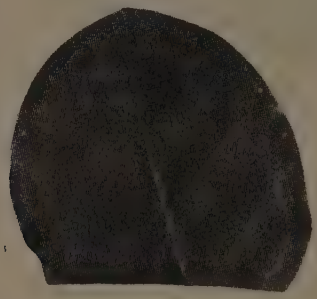

Fig. 6. Local-lesions on *Nicotiana tabacum* var. White Burley.

Brassica rapa (turnip var. Purple Top White), *B. napus*, and *Hesperis matronalis* carried the virus symptomlessly.

In case of the following cruciferous species when inoculated mechanically as well as through the aphid vector, *Brevicoryne brassicae*, neither any visible symptoms were observed nor the virus could be recovered back in sub-inoculations on *B. nigra: Brassica oleracea* L. var. *acephala* DC. (kale), *B. oleracea* L. var. *botrytis* L. (cauliflower), *B. oleracea* L. var. *capitata* L. (cabbage), *B. caulorapa* Pasq. (kohl-rabi or knol khol), *B. oleracea* L. var. *gemmifera* Zenker (Brussel's Sprouts var. Cambridgeshire Champion), and *Raphanus sativus* L. (radish; three commercial varieties including French Breakfast and Scarlet Globe were tested). Also, the virus could not be transmitted by sap-inoculation to any of the following plant species: *Nicotiana glutinosa* L., *N. rustica* L., *Solanum nigrum* L., *Datura alba* Nees., *D. stramonium* L., *Petunia hybrida compacta* Vilm. Var. Rosy Morn.; *Cucumis sativus* L. vars. White Spine and Paris Early, *C. melo* L. var. *utilissimus* Duthie and Fuller; *Amarantus blitum* L., *A. tricolor* L., *Celosia plumosa; Anchusa capensis; Phaseolus vulgaris* L. var. Canadian Red., *Vigna sinensis* Savi., *Vicia faba* L., *Lathyrus odoratus* L.; *Papaver* sp.; *Callistephus chinensis* Nees., and *Dahlia* sp.

Properties of the Virus. All tests were carried out on young and healthy plants of *Brassica juncea* var. *rugosa* and *B. nigra* which were found to be most suitable.

(i) *Thermal Inactivation:* The virus was found to withstand ten minutes exposure to 52°C but not 55°C at which temperature it was rendered innocuous.

(ii) *Dilution End Point:* The undiluted leaf-extract from diseased plants of different ages remained infective in dilutions upto 1 : 1,000 but could not withstand dilution of 1 : 3,000.

(iii) *Longevity in Vitro:* It was found that the infective sap stored during Jan. - Feb. at room temperature (about 10–15 C) started losing infectivity after 144 hours of storage. In many tests at the same temperature, however, it was found to show some infectivity upto 216 hours of storage but no infection at all was obtained after it had remained stored for 240 hours.

Insect Transmission. The virus was transmitted by three species of aphids, *Aphis gossypii*, *Brevicoryne brassicae*, and *Myzus persicae*. The virus appears to be of non-persistent types as the viruliferous aphids, *B. brassicae*, when transferred to healthy plants of *Brassica nigra* immediately after acquisition, lost the virus within 2 hours of feeding on healthy plants.

Detailed investigations on virus-vector relationship in respect of the aphid, *B. brassicae*, have been carried out and are being reported separately.

Discussion. Sylvester in 1953 describing the host-range and properties of *Brassica nigra* virus listed 25 other distinct viruses and virus strains recorded in Cruciferae in the aphid-transmissible group of viruses

alone. In addition, there is the turnip 'yellows' virus reported from Belgium (Vanderwalle and Roland, 1951) which though not sap-transmissible is also an aphid-borne virus. Smith (1957) in his recent treatise on plant viruses, has recognized only eight viruses of Cruciferae. The viruses affecting cruciferous crops in Great Britain have recently been reviewed by Broadbent (1957).

The virus under report appears to have resemblance with the virus reported from Trinidad by Dale (1948), which also seems to be the only previous record of a virus naturally occurring on *Brassica juncea*. However, the two viruses show some significant differences in host-range, e.g., Dale's virus is transmissible by sap-inoculation to turnip whereas the virus under report could infect this host only through the aphid vector, *Brevicoryne brassicae*, and all attempts of transmission by sap-inoculation remained unsuccessful. Also, the virus reported from Trinidad is transmissible to *Raphanus sativus* var. *hortensis*, whereas the virus under report could not be transmitted to any of the three varieties of *R. sativus* tested either by sap or aphid-inoculations. In some of its properties the virus investigated also resembles the virus reported from China by Ling and Yang (1940) which, however, being transmissible to radish and Chinese radish and having a dilution end point of 1 : 6,000 is obviously different. A turnip mosaic virus reported by Tompkins (1938) has also some properties in common with the virus investigated; but that virus is quite different because of its transmissibility to cabbages or 'coles', and it has been described by Smith (1957) under Cabbage Black Ringspot Virus.

Although most of the aphid-borne crucifer viruses show differences from one another in their host-range and other properties, Walker *et al* (1945), and Pound and Walker (1945) have classified them into two groups, viz., the Turnip virus 1 and Cauliflower virus 1 groups. The later workers have mostly followed this classification. Sylvester (1953) has recently proposed the addition of a third group named as Radish virus 1 group.

Considering the characteristic properties of the Chinese *sarson* mosaic virus under report, viz., severe blotchy mottle and malformation of leaves in some mustards e.g., *Brassica nigra*, systemic infection in hosts outside Cruciferae, e.g., *Zinnia elegans*, thermal inactivation between 52–55 °C. dilution end point between 1 : 1,000 – 1 : 3,000 and longevity *in vitro* for about 9 days, it falls in the Turnip virus 1 group. Also, the virus cannot be identified with any of the eight crucifer viruses recognised by Smith (1957).

Further, the virus is non-persistent in its aphis vector, *Brevicoryne brassicae*, in accordance with the classification suggested by Watson and Roberts (1939, 1940).

In host-range tests, turnip var. Purple Top White has shown an interesting reaction since this host could be infected only through the insect-vector and not by sap-inoculation. A similar reaction has been reported on certain hosts in case of *Brassica nigra* virus (Sylvester, 1953)

which also belongs to Turnip virus 1 group. Also, a significant feature of host-range of these viruses is that in the genus *Brassica* they are transmissible only to the species belonging to 'mustard' group and not to any of the 'cabbage' or coles'. However, the virus under study differs from the *Brassica nigra* virus in physical properties and its ability to infect *Nicotiana tabacum*.

Very little information is available on the virus diseases of crucifers from India where a large number of cruciferous crops are extensively grown under varying climatic conditions. McRae (1924) reported the occurrence of mosaic in radish (*Raphanus sativus* var. *caudatus*) in Bombay area. A mosaic disease of radish, occurring in Delhi area, was also recently investigated by Raychaudhuri and Pathanian (1955).

The occurrence of the virus reported herein deserves serious attention as it has been shown to severely affect most of the oil-producing Brassicas which occupy a very important place in the agricultural economy of the country.

Summary

A severe mosaic disease of Chinese *sarson* (*Brassica juncea* var. *rugosa*) occurring in Simla Hills has been studied.

The causal virus is sap-transmissible. Three species of aphids, viz., *Aphis gossypii*, *Brevicoryne brassicae*, and *Myzus persicae* have been found to be the vectors of the virus.

The virus has been transmitted by sap-inoculation as well as through the agency of the aphid vector, *B. brassicae*, to a variety of host plants in Cruciferae. In addition, it has been transmitted by sap-inoculation to *Zinnia elegans* and *Nicotiana tabacum* outside Cruciferae, causing systemic infection in *Z. elegans* and local-lesions on *N. tabacum*. In the genus *Brassica*, however, the virus is transmissible only to the species belonging to the 'mustard' group and not to any of the 'cabbages' or 'coles'.

The virus is inactivated between 52–55 °C. by 10 minutes exposure and has a dilution end-point between 1 : 1,000 – 1 : 3,000 and survival *in vitro* for about 9 days. The virus belongs to Turnip virus 1 group and is of non-persistent type.

Acknowledgements. The authors are highly grateful to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, I.A.R.I., New Delhi for his valuable guidance, during the course of the investigation. Grateful thanks are also due to Mr. S. Kanakaraj David, Assistant Entomologist, Agricultural College and Research Institute, Coimbatore, for kindly identifying the aphids.

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MUTATION IN COLLETOTRICHUM FALCATUM WENT, THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE. II. INDUCED BY FAST NEUTRONS

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(Accepted for publication March 15, 1959)

Earlier studies have shown that there is a great deal of variation both regarding pathogenicity and cultural characters in *Colletotrichum falcatum* Went, the causal organism of red rot disease of sugarcane. The development of new pathogenic strains in nature is often reported and has resulted in failure of several varieties once considered to be resistant to the red rot organism and has often upset the entire varietal set-up of certain sugarcane tracts in the country. Studies on the nature of variation in this organism are, therefore, of fundamental importance with a view to obtain information on the conditions under which new forms are arising in nature. In these studies, however, only mutations induced artificially with the aid of fast neutrons are reported. A mutant of *C. falcatum* obtained with the aid of radioactive phosphorus (P^{32}) which showed comparatively shorter spores with marked change in their shape has already been reported (Vasudeva *et al*, 1957 & 1958).

Fast neutrons are considered to be good mutagenic agents and are more efficient than X-rays because they produce relatively higher proportion of chromosome aberrations (Giles, 1943; Thoday, 1942) and have comparatively greater penetrating power due to absence of electrical charge (MacKey, 1951). A heavy spore suspension of single-spore culture of the light and virulent isolate No. 244 (Indian Type Culture Collection) of *C. falcatum* was prepared in sterile distilled water and washed twice by centrifugation under aseptic conditions. Equal volumes of the suspension were exposed for 10 minutes, 1, 2 and 4 hours to fast neutrons produced through the Cascade Generator (using beryllium as target) at the Tata Institute of Fundamental Research, Bombay in 18 mm. diameter pyrex glass test tubes. The suspension tubes were arranged around the target at 3 cm. distance in such a fashion that each tube received a neutron flux of 0.35×10^7ns./cm^2./sec. The tubes were chilled periodically during the exposure and the irradiated cultures were stored at 7 °C. to avoid germination of spores. The control tubes were also maintained under similar conditions.

Screening of Mutants. The treated as well as untreated spores were plated on oat meal agar* by Dilution Pour Plate Method and were allowed to grow. After 48 hours of incubation at room temperature (15-19 °C), 744 distinct single spore colonies were picked up on oat meal

*Quaker Oats 40 gm.; Agar Agar 20 gm.; Dist. Water 1 litre.

agar slants for screening mutants. In addition, 2,393 colonies were examined in the plates themselves as they had formed distinct zones around the place of the origin and subcultures made where found necessary.

All the single spore cultures when fully grown were examined and found to resemble the parent type both microscopically and macroscopically except one each in the series exposed for 10 minutes and two hours. The former showed black spore masses which were intermixed with normal pink ones. Spore shape and size in both the cases were found to be normal. Subcultures made from both the categories of spore masses produced pink masses in the subsequent generation resembling the parent type. The culture picked up from the population irradiated for 2 hours showed uniform distinct creamy-white spore masses on the entire surface of the slant instead of the characteristic pink ones in the parent type. The appearance and all other cultural characters including shape, size and the granulation of the spores of the mutant were almost similar to those of the parent except the suppression of the pigment in the former. The mycelium in the initial stages was also suppresssed and silky like the parent type.

Further mass as well as single spore cultures were made on oat meal agar and carried through 15 more generations without any tendency of reversion to the parent type.

Morphological and Physiological Studies. In order to see physiological differences, if any, between the mutant and the parent culture and to determine the influence of nutrition on pigmentation, they were grown on dextrose-asparagine-phosphate (D.A.P.) medium*; D.A.P. supplemented with 0.01% Bacto yeast extract; D.A.P. plus 0.5% vitamin-free acid hydrolyzed casein; a complete medium containing D.A.P., yeast extract, casein and trace elements**; oat meal agar; potato dextrose agar*** and 10 per cent cane juice agar. The mutant showed good growth and formed creamy white spore masses on all the media used but in case of yeast extract the substrate had turned black and mycelial growth was also comparatively less cottony. In case of casein hydrolyzate there was profuse cottony mycelial growth and the colour of the substrate had turned black only at the point of inoculations. The parent culture also showed similar characters on all the media except for the presence of pink pigment of the spore masses. Of all the media tried, oat meal agar gave the best growth and sporulation as in case of the parent culture.

The parent culture (isolate No. 244) was grown on liquid medium (D.A.P.) in 100 ml. flasks at room temperature (17-20°C.) and after 10 days' growth the mycelium was filtered and culture filtrate collected. The former was dried in a desiccator and ground with equal amount of

*Dextrose	30 gm.	**H_3BO_3		***Peeled Potatoes	250 gm.
Asparagine	1.0 gm.	$CuSO_4$. $5H_2O$		Dextrose	90 gm.
KH_2PO_4	1.5 gm.	MoO_3 (85%)		Agar Agar	20 gm.
$MgSO_4$	0.5 gm.	$MnSO_4$. $7H_2O$		Dist. water to make 1000 ml.	
Agar Agar	20.0 gm.	$ZnSO_4$. $7H_2O$			
Dist. water to make 1000 ml.		$FeSO_4$. $7H_2O$			

pure sea-sand and subsequently mixed with the latter. This was centrifuged and filtered through pyrex brand sintered glass filter having an average pore diameter of 1.4μ. One ml. of the culture filtrate was added to 10 ml. of the autoclaved liquid medium (D.A.P.) in 100 ml. flasks. The mutant was grown on this medium to see the effect of culture filtrate and enzymes of pink spore forming strain on its sporulation. The mutant, however, showed normal growth and sporulation but the spore masses remained creamy-white.

Spores of the mutant as well as parent culture of the same age were washed twice by centrifugation and put for germination in glucose (1%), sucrose (1%), yeast extract (0.1%) and distilled water. The following data on germination, recorded after 17-19 hours' incubation at 25°C., showed that the germination percentage was comparatively less in case of mutant than in the parent type.

Substrate	Per cent germination	
	Mutant	Parent
Sucrose	4.8	21.3
Glucose	3.0	9.5
Yeast extract	23.9	68.2
Dist. water	0.9	4.5

An experiment was also set up to determine whether perithecia could be produced in case of the mutant culture under laboratory conditions. Since the capacity of the parent culture (Isolate No. 244) to produce perithecial stage under laboratory conditions was doubtful, isolate No. 388 (originally isolated from sugarcane variety Co. 527) of the Indian Type Culture Collection was also used to serve as control. The technique applied for the purpose was same as reported by Carvajal and Edgerton (1944) and Chona and Srivastava (1952). It was observed that neither the parent culture nor the mutant formed ascigerous stage on the old dry autoclaved sugarcane leaves. In case of isolate No. 388, however, abundant perithecia were produced under similar conditions.

Pathogenicity. In order to test the pathogenicity of the mutant as also its stability on the natural substrate, 50 canes of sugarcane variety, Co. 647, were inoculated by the standard Plug Method described by Chona (1954). Similarly another lot of 50 canes of the same variety were also inoculated with the parent strain under identical conditions to serve as control. Care was taken to irrigate the two sub-plots independently in order to avoid any mixing of strains. The infection was allowed to progress for about 3 months with periodic observations and the final observations were recorded by splitting the canes longitudinally and measuring the linear spread of infection of red rot. The results of such an experiment showing the comparative virulence of the mutant and parent (No. 244) are set out below.

Strain	No. of canes inoculated	No. of canes examined	Linear spread of infection in cm.		
			Minimum	Maximum	Average
Mutant	50	37	33.00	88.90	56.90
Parent	50	43	27.90	68.60	47.95

The inoculated canes showed typical reddening of the pith with white cross bands and characteristic alcoholic smell in both the cases. A large number of isolations were made on oat meal agar from different portions of several canes inoculated with both the strains. All the isolations in case of the mutant yielded cultures with creamy-white spore masses resembling the mutant strain. Likewise, the isolations of the control yielded normal cultures characteristic of the parent type.

Discussion. The colour mutant screened through a large irradiated population represents a stable change as it could be carried through a number of asexual generations and could be re-isolated in its typical form even after it had parasitised on its natural substrate, i.e. sugarcane, under natural field conditions for nearly three months.

The pigments in fungi are supposed to be determined in part by genetic factor and in part by environment including nutrition (Lilly and Barnett, 1951). Among the nutritional factors micro-essential elements are known to exert comparatively greater influence on pigment formation. Change in the nutrition of the mutant on artificial culture medium, including addition of trace elements, did not have any effect on its characteristics, showing, thereby, that the expression of the pigment is not influenced by any nutritional factor in this case. The culture filtrate as well as the mycelial extract of the parent culture, incorporated into the medium, did not have any effect on the pigment of the spores of the mutant which indicates that the change is neither controlled by enzymatic action nor the metabolic products of the parent culture have any effect on restoring the pigmentation of the mutant. Considering all the facts stated above, the possibility seems to be that the change induced through the bombardment of fast neutrons might be controlled by a genetic factor which has resulted in a stable colour mutant.

It is evident from the results of pathogenicity tests that the mutant has not suffered any loss in pathogenicity and produces sufficiently good infection on sugarcane comparable to that of parent culture. The symptoms produced by the mutant on the host were characteristic of red rot disease. Thus the suppression of the pigment that gives the spore mass a pink colour in normal cultures, seems to have no detrimental effect on the host-parasite relationship including virulence. The data on germination of spores of the mutant and the parent culture, as studied on different substrata, show that the germination capacity of the spores of the parent under similar set of conditions is comparatively higher but it is difficult to say whether it is influenced with the presence of pigment in the spores.

In fungi the function of pigments especially those located only in the spores is not well understood though in some cases these have been assumed to serve a definite purpose.

Summary

A mutant was picked up from the population of *C. falcatum* spores irradiated with neutrons for 2 hours which forms creamy-white spore masses instead of the characteristic pink ones normally produced in the parent culture. The mutant could be carried through a number of generations without any change in its morphological characteristics. Inspite of the absence of the pigment which gives pink colouration to the spore masses in the normal cultures, the mutant not only produced characteristic symptoms of red rot disease on sugarcane variety Co. 647 but gave as good infection as the parent culture. It could also be re-isolated in its typical form from the inoculated canes.

The germination percentage of the conidia of the parent was found to be higher than that of the pigment-less mutant. Like its parent, the mutant does not form ascigerous stage under laboratory conditions. Nutritional experiments have indicated that the absence of the pigment in the spores neither seems to be controlled by nutritional deficiency nor enzymatic action. The change induced by fast neutrons is, therefore, considered genetical resulting in a stable mutant.

Acknowledgment. Our sincere thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his valuable guidance and helpful criticism and Dr. B. L. Chona, Mycologist, for his keen interest and suggestions throughout the course of this work. The authors are also grateful to Dr. H. J. Bhabha, Chairman and Dr. R. Ramanna, Principal Research Officer, Atomic Energy Establishment for providing facilities for irradiation of samples.

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HETEROTHALLISM IN PUCCINIA CARTHAMI (HUTZ.) CORDA, THE RUST OF SAFFLOWER

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Introduction. Since the discovery of sex in *Puccinia graminis* and *P. helianthi* by Craigie (1927, 1933) considerable amount of work has been done by different investigators on the occurrence of this phenomenon in several other rusts. Reviewing this work from 1927 to 1942, Buller (1950) reported that out of 26 rusts studied 22 proved to be heterothallic. From the practical point of view the study of sex in rusts is important in so far as it explains the variability in these fungi which otherwise do not commonly mutate in their pathogenic behaviour.

The life-history and perpetuation of safflower rust in India were studied by Prasada (1947) and Prasada and Chothia (1950). They found that this rust is perpetuated through oversummering teliospores. Since the rust is autoecious, there is a strong probability of the formation of new physiologic races if it were shown that it is heterothallic. These studies were, therefore, undertaken to throw light on the sexual behaviour of this rust and the results obtained are given here.

Material and Methods. Before attempting to infect safflower seedlings with sporidia it was necessary to secure good and consistent germination of teliospores. It has been shown by Prasada and Chothia (1950) that two types of teliospores are formed in this rust. Some of them germinate soon after formation, while others require a resting period of 5 to 6 months. It is also known that teliospores formed on the crop in April do not lose their viability inspite of the summer heat.

Infected leaves bearing telia were collected from the crop in April and stored carefully in a cool place during summer. A few pieces of leaves were soaked in water at frequent intervals and teliospores from them were put for germination on glass slides in moist chamber at 18°–20°C. Good germination was secured by this method in December, and this material was used for inoculation.

Seedlings were raised in 6 inch pots, and plants 15 to 20 days old were used for inoculation. Before inoculation the pots were kept in humid chambers and gently sprayed with water by an atomiser. Inoculations were generally made in the afternoon. Viable teliospores were taken from previously soaked leaves and kept for germination on glass slides in humid chambers about 96 hours earlier at 18–20°C. as they generally took that much time for germination and formation of sporidia. For inoculation, germinating teliospores were transferred on the upper surface of the leaves with the help of a loop needle. The inoculated pots were further incubated in moist chambers for 48 hours before they were put on greenhouse bench.

In another method of inoculation, the atomised seedlings were covered with a lantern globe on which one Petri plate, lined with moist filter paper, was used as a lid. Just above the seedlings were suspended telia-bearing leaves previously soaked in water. The lid was removed at frequent intervals and a gentle spray of water was given to maintain high humidity. The teliospores on germination shot out sporidia and a well distributed infection was obtained all over the covered plant.

The infected material at different stages of development was fixed in Formalin-acetic-alcohol, properly dehydrated, embedded and microtome sections 10μ thick were cut. The sections were stained in the following combinations:—

a. Heidenhain's Iron alum haematoxylin counterstained with fast green.

b. Conant's quadruple stain.

Experimental. Usually 7 to 8 days after inoculation with germinating teliospores, pale green flecks appeared on the leaves which proved to be pycnia. In another 3 to 4 days uredia appeared either on one or both surfaces of the leaves. In some leaves, however, it was observed that uredia were not formed even after a fortnight and these centres of infection remained sterile. Both the fertile and sterile infections were studied by taking free hand and microtome sections.

Sections taken through fertile and sterile regions showed well-developed pycnia on one or both surfaces of the leaves. Pycnia were invariably sub-epidermal, flask-shaped or spherical, and measure 80–100 μ in diameter. A large number of flexuous hyphae were found protruding out of the ostioles. The pycnia also produced numerous pycniospores which were seen oozing out through the ostiole. Sections taken through the fertile regions showed profuse development of intercellular mycelium in the palisade and spongy parenchyma of the leaf (Text Fig. 1). The uredospores were found to develop on both sides of the leaves usually near the pycnia. In some cases, as shown in text fig. 2, uredia were formed in between two very closely situated pycnia.

On the other hand, sections taken through 25–30 days old sterile infections showed that further development of the mycelium and consequently, the formation of uredospores had been completely suppressed even when the pycnia were side by side and there had been free intermixing of the nectar (Plate I & II). The intervening wall of the compound pycnia had disintegrated and their contents had become intermixed, while in the other the pycniospores of one had been mixed with those of the other, yet both of them remained sterile. This indicates that in this rust also probably two strains (+) and (—) exist which must be brought together to bring about fertilization and formation of uredospores.

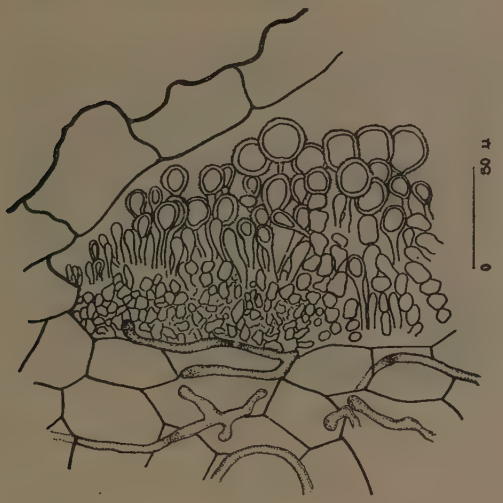


Fig. 1

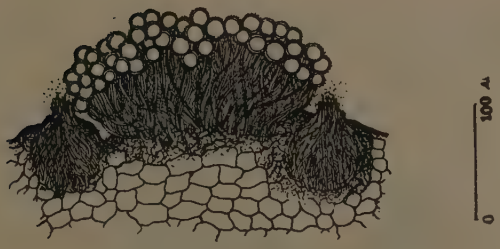


Fig. 2

In order to confirm this, another set of inoculations was carried out by the second method in which the plants were put under a lantern globe and leaves infected with viable teliospores were suspended from above. Sporidia were shot out from teliospores on germination and widely separated centres of infection were obtained. Eight single sporidial infections on eight different plants were selected. These were marked as A, B, C, D, E, F, G and H by tags. The mixing of nectar was done as follows:

(i) Nectar of A and B was gently stirred with a sterilised needle and the plants were kept separately. No uredia were formed.

(ii) The nectar of pustule C was mixed with all the remaining pycnia, viz., D,E,F,G, and H. After each transfer the needle was flamed to eliminate all chances of contamination.

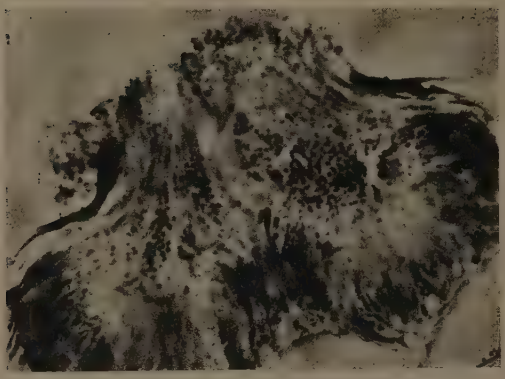


Plate I

Plate II

It was observed that 4–5 days after the mixing of nectar the development of uredospores proceeded in the normal way in pustules D, G and H, whereas in the two remaining cases E and F uredospores did not appear even after a week. This shows that pycnia marked C, E and F were of the same strain and consequently failed to produce uredospores, while those marked D, G and H were of different strain from that of C and hence proved to be fertile.

In order to confirm these results, a large number of leaves of a susceptible variety of *Carthamus tinctorius* were inoculated with germinating teliospores by the loop needle method in a chamber of the greenhouse and observations were recorded periodically. Final counts of fertile (with uredia) and sterile (without uredia) infections were taken after sufficient interval. The data of such an experiment are presented in Table I.

TABLE I. Inoculation of safflower leaves with germinating teliospores, and formation of fertile and sterile mycelium

Date of Inoculation	Date of appearance of pycnia.	Date of observation.	Number of pustules with uredia	without uredia	Total
6 Feb., 1956	15 Feb., 1956	20 Feb.	23	14	37
		28 Feb.	23	14	37
		6 March	23	14	37
		14 March	25	11	36*
		18 March	25	11	36
11 Feb., 1956	20 Feb., 1956	24 Feb.	26	26	50
		28 Feb.	26	24	50
		10 March	27	22	49*
		22 March	27	22	49

* One leaf died

A careful study of Table I shows that when the final observations were recorded nearly a month after the appearance of pycnia, the number of fertile pustules increased in two cases. Craigie (1927) had also observed that in *Puccinia helianthi* the number of fertile pustules increased by keeping them longer under observation. In order to explain this change he stated that two components, although of opposite strains, were at first widely separated (about 4 mm.) and consequently the interaction of their mycelia was delayed. Some such thing must have happened here too by which the number of fertile infections increased with longer incubation.

Diplodization in single sporidial infections and formation of 'Uredinoid aecia': Some single sporidial infections on safflower leaves were kept in a separate glass house where no other culture of this rust was being maintained. It was observed that in these cases the normal development of uredia did not take place, but after about 20 days, small, black, pinhead-like dots appeared near the edges of pycnia on the lower side of

the leaves. Within a few days these spots enlarged and the dots became more prominent. Transverse hand sections through this region showed the presence of smooth-walled round spores, very much resembling the uredospores. To study the exact mode of their formation, and their morphology, material was fixed in Formalin-acetic-alcohol and microtome sections were cut at 10μ thickness. Sections were stained in Heidenhains Iron alum haematoxylin, counter stained with fast green, and in Conant's quadruple stain. In the infected region distinct anatomical changes such as reduction in the thickness of the leaf and disorganisation of pallisade and spongy parenchyma were observed (Plate III). The flexuous hyphae in the pycnia too had degenerated. Small saucer-shaped sori without peridium or paraphyses were formed on both sides of the leaves. In these sori were seen spores which were round or spherical, 21–25 μ in diameter, with perfectly smooth walls unlike the normal uredospores, and borne terminally on a thick hyaline pedicel arising from dikaryon cells. Quite a few of them were formed in chains, they were produced in basipetal succession just like the aeciospores. The mature spores and their primordia were binucleate (Plate IV & Fig. 16, 17). These types of spores have been called 'Uredinoid aecidia' by Christman (1907), Arthur (1929) and others. Significance of their formation in brachyform rusts will be discussed later.

In the earliest stages of development tangled weft of hyphae appeared below the epidermis in these sori (Text fig. 3). The hyphae enlarged gradually and formed a dense mass of cells which gave the appearance of pseudoparenchyma (Text Fig. 4). The cells were of the same size, irregularly polygonal in shape and uniformly uninucleate (Text fig. 5). As these sori advanced in age the uninucleate polygonal cells got diplodized. To achieve this, intervening wall between the adjacent cells lying side by side was partially dissolved (Text Figs. 6 & 7). The fusing cells elongated in size and their nuclei came together in conjugate pairs in the two-legged cells so formed (Text Figs. 8 & 9). Gradually, the cell enlarged in length and the cytoplasm and nuclei aggregated near the tip region. Later on, a septum developed cutting the tip region from the rest of the cell. The tip grew in size and ultimately developed into the spore (Text figs. 10–12). It was also observed that at places where the spore initials were compactly placed, some of them gave false appearance of being bicelled spores due to pressure from both sides (Text 13–15).

Conclusions and Discussion. The experiments described above clearly show that *Puccinia carthami* is heterothallic. Inspite of mixing the nectar with a sterilized needle several pycnia remained sterile. Similarly, several compound pustules formed as a result of two sporidia causing two infections close to each other did not develop further. The only explanation of the occurrence of these sterile infections is that in this rust also, like *Puccinia graminis*, teliospores on germination produce two sporidia of one sex and two of the other. When two pycnia of different sex are formed very close together forming a compound pustule or pycniospores of different sex intermix, the infection proceeds normally and uredia are formed, otherwise the infection remains sterile if they are of the same sex. As to be expected, approximately half the number of

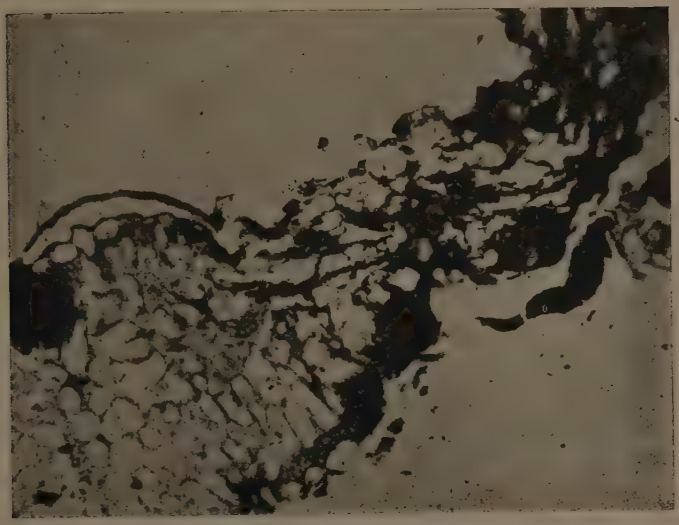


Plate III

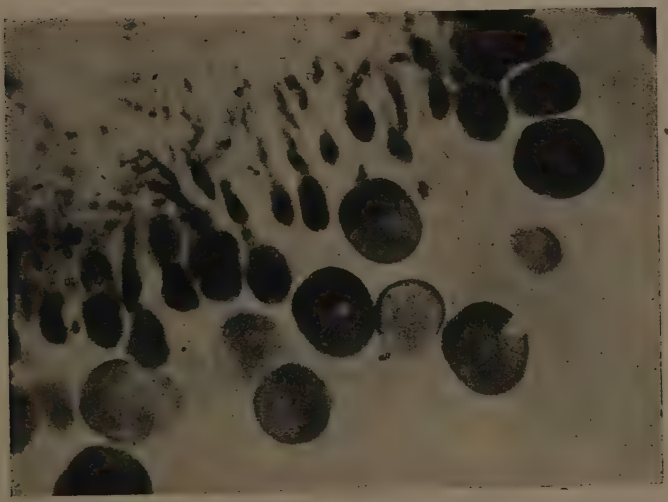
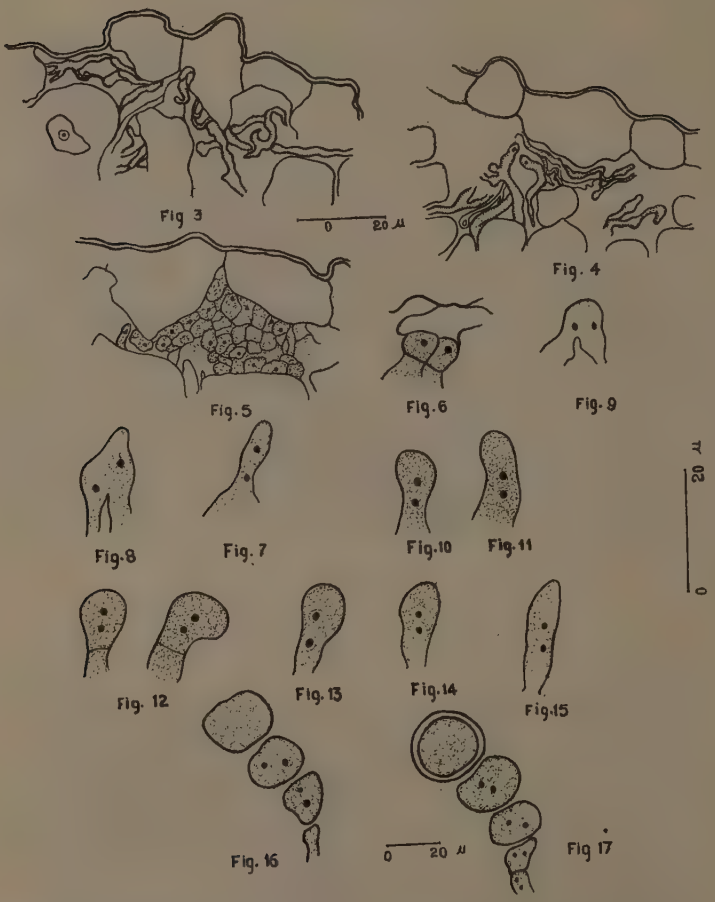


Plate IV



pustules are fertile since there is an even chance of sporidia of like and unlike sex coming together.

Since it is an autoecious rust and also has been shown to be heterothallic, there is every likelihood of the formation of new races by hybridization in nature. This aspect, which is of great practical value specially in connection with the breeding of rust resistant varieties, requires attention.

An interesting finding which has emerged from these studies is the formation of binucleate, smooth-walled spores, in chains and cut in basipetal succession like the normal aeciospores. Normally, this rust

is brachyform and aecial stage is missing. But, in some cases, where single sporidial infections were kept undisturbed for 20–30 days after the formation of pycnia, aeciospore-like spores, referred to above, were produced. In literature such spores have been named as 'Primary uredospores' or 'Uredinoid aecidia' because of their position in the life-cycle of the pathogen and also due to their morphological resemblance to aeciospores. Formation of such spores has always aroused interest because of their rare occurrence. They have been differently interpreted by various investigators. Christman (1907) pointed out that the sorus commonly called as 'primary uridia' was in fact morphologically an aecium. According to Arthur (1929) certain aecia with spores borne singly on pedicels and usually without peridium or paraphyses are stylosporic or uredinoid aecia but were still referred to as 'primary uredia'. He was of the opinion that because of their place in the life-cycle of the species in which they occur, together with their mode of origin, they might be considered as true aecial stages. Reviewing the life-cycles of rusts Bessey (1952) has stated that in the brachy-form rusts usually designated by the formula 0, II, III, if pycnia were succeeded by sori containing spores resembling the typical uredospores which were designated by Christman (1907) as 'uredinoid aecia', they should be interpreted as 'Macrocyclic rusts' of 0, I, II, III, formula.

Cytological investigations show that in all cases the primordial cells in the 'uredinoid aecidium' were uninucleate, and their diploidization was achieved by Christman type of cell fusion. The spore mother cells were all binucleate. Allen (1932) studied the cytological behaviour of the single sporidial (unisexual) infections in *Puccinia coronata* and found that mostly multinucleate cells were formed at the later stages and the spores produced were also multinucleate. She remarked, "The formation of such spores was a futile expression of the reproductive tendency". In *Puccinia carthami* the regular binucleate condition observed in these spores and also in their primordia show close similarity with the normal aeciospores and lend further support to our view that in the light of these findings this rust could probably no longer be called a brachyform or microcyclic rust.

Acknowledgement. Sincere thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his keen interest and helpful suggestions.

Summary

Controlled laboratory and greenhouse experiments have shown that *Puccinia carthami* is heterothallic.

The formation of 'Uredinoid aecia' from single sporidial infections has been demonstrated. Cytological studies show regular binucleate condition in spores formed in 'Uredinoid aecia' as well as in their primordia just like the normal aeciospores. Results are discussed in detail to show that this rust could probably no longer be called brachyform or microcyclic.

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STUDIES IN PUCCINIA HORDEI OTTH., THE LEAF RUST OF BARLEY, IN INDIA

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In India, barley is cultivated mostly in the Northern plains particularly in the States of Uttar Pradesh, Rajasthan and Bihar. Like wheat crop it is sown in October and November and harvested just ahead of wheat. The crop is commonly affected by black rust (*Puccinia graminis tritici* (Pers.) Erikss. & Henn.) and yellow rust (*Puccinia glumarum* (Schmdt.) Erikss. and Henn.). The leaf rust or the dwarf rust of barley (*P. hordei* Otth.) is very rarely found and has, therefore, not received much attention by earlier workers though its occurrence in India was recorded by Butler as early as 1918. Apparently no work on this fungus has been done in this country. It has, however, received more attention in other countries like U.S.A. (Mains, 1926 & 1930), Australia (Waterhouse, 1927 and 1929), Canada (Brown, 1931), Germany (Hey, 1931), Argentina (Hirschhorn, 1933) and Portugal (D'oliveira, 1937 and 1939) where it is of common occurrence.

It was during 1954-55 crop season that some experimental plots of barley grown for trials at the Indian Agricultural Research Institute, New Delhi showed a fairly heavy infection of barley leaf-rust. Samples were also collected from other parts of the country in the same season. In all, five collections from cultivated barleys were obtained from Delhi, Uttar Pradesh and Madras State from March, 1955 to October, 1957. The cultures were maintained on a local barley (*Hordeum vulgare*) and on variety 'Spratt Archer' (also used as control by D'oliveira, 1939).

The Uredo Stage : Under Delhi conditions the rust makes its appearance usually in the month of February or March. First of all uredia appear usually on leaves and culms and they are paraphysate, irregularly scattered on both surfaces of the leaf and citron-yellow or light brown in colour. Uredospores are roundish or ellipsoidal, yellow, 19 to 22 μ in diameter when round, and 22–26μ x 15–20μ when ellipsoidal. Spore wall is thick, echinulate; germpores numerous and scattered.

Fresh uredospores, if placed on a thin film of water, start germinating in 45 minutes at 17°C. and the maximum germination is reached after 6 hours. 55–65 per cent spores germinate at 3°–6°C., 65–75 per cent at 10°–13°C., 80–90 per cent at 16°–18°C., 60–70 per cent at 21°–23°C., and 5–15 per cent at 26°–28°C. No germination has either been observed at 1°C. or at 33°C. The optimum range, therefore, lies between 16°–18°C., which compares favourably with that obtained with leaf rust of wheat by several workers. Uredospores when frozen for 24 hours in the freezing

chamber and transferred to a thin film of water at the optimum range (16°–18°C.) started germinating normally within an hour.

Spores kept for 4 hours at 35°C. in a thin film of water and then transferred to 16–18°C showed 60–70 per cent germination but no germination was observed when the spores were kept for 24 hours at 35°C. Spores kept dry in butter-paper packets and exposed to as high as 45°C. for 24 hours showed only traces of germination (upto 3 per cent). Distortion of the germ tubes was observed at temperature ranges higher than the optimal. Thus, at temperature ranges unfavourable for spore germination the uredospores of this rust are not killed for a number of hours.

A number of factors, chiefly temperature and moisture influence considerably the longevity of spores under storage. According to Barclay (1891) uredospores of some rusts in Himalaya retain viability from two to eight months after collection. In case of cereal rusts, it has been observed by many workers (Chester, 1946; Schilcher, 1932; Stroede, 1934) that uredospores remain viable from 2 to 12 months under different conditions of temperature and humidity.

Uredospores can remain viable upto nine months (37 weeks) if, stored dry at low temperatures soon after formation in dry and moderate season. Uredospores collected from two test cultures in the month of May (temperature range of 6°–27°C) at Simla gave upto 5 per cent germination at the end of 37 weeks when stored dry in refrigerator at 0°–5°C. Shorter periods of storage consistently gave progressively higher viability, notwithstanding their different dates of collection in drier and moderately warmer month of June to less dry and much cooler month of February. Uredospores kept in butter-paper packets in a room (temperatue range of 9°–14°C.) between December to April at Simla showed 25–30 per cent germination when tested after four months.

THE TELEUTO STAGE: Teleuto-sori are confluent, lead-black, scattered on leaves and culms, and covered by the epidermis. Both unicelluar (meso) and bi-cellular (teleuto) spores often separated by brown paraphyses, are observed in the same sorus. Spores are thick and smooth-walled, oblong-clavate in shape, often pear-shaped, apex rounded, flattened or drawn out to one side, chestnut brown in colour, 25–50 x 15–25 μ and short stalked. Attempts to germinate the teleutospores have so far not been successful.

PHYSIOLOGIC SPECIALISATION: Though the presence of physiologic races in this rust was first demonstrated by Mains as early as 1926, apparently no attempt was made to use a standard set of differentials till D'oliveira (1939) proposed a selection of differential hosts for the differentiation of physiologic races of *P. anomala* (*P. hordei*) on cultivated barleys. Levine and Cherewick (1952) selected a set of sixteen differentials and on the basis of reactions of them they have recorded 52 races from various countries.

During the course of these studies the symbols used by Levine and Cherewick (1952) have been followed. Inoculations were made in the usual way under spore proof conditions and reactions were recorded twice; first within 3–5 days after the rupture of the pustules and second, seven days later. It has been observed that unlike *Puccinia triticina, P. hordei* shows pronounced chlorosis or necrosis with the advance of time.

For want of seeds of recognised differential hosts at the time of starting the work, two hundred and eight barley varieties comprising of indigenous varieties of *Hordeum vulgare* L. and *H. distichon* L. and also various exotic material (Afghanistan, Nepal, United Kingdom, Europe United States, Australia and other countries) were tested with the five isolates (2 from Delhi and one each from Raya, Kanpur and Ketti) of barley rust and tests were repeated through different seasons. On the basis of these tests, 126 varieties were found susceptible, 16 resistant and 63 showing variable reactions to all the five isolates; whereas well marked and consistent differences were found on the remaining 3 varieties. Details of reactions of these 3 varieties are given in Table 1:—

TABLE 1. Reactions of three barley varieties to 5 isolates of *Puccinia hordei* Otth.

Isolate No.	Locality	Original host	Name of Barley Varieties				Remarks
			B. 4	Afghanistan – 12	Afghanistan–9–12	Control	
1.	Delhi	*Var.* unknown	4	4	4	4	Form 1
2.	Delhi	*Var.* unknown	4	4	4	4	
3.	Raya	Bar. K. 3	0	0	0	4	Form 2
4.	Ketti	*Var.* unknown	0	0	0	4	
5.	Kanpur	Bar. Selec. 54	0	0	0	4	

On the basis of these reactions the five isolates fell into two groups as indicated above in the remarks column.

When seeds of differential varieties, used by Levine and Cherewick (1952) was received and tested, it was discovered that all the five isolates produced identical reactions on sixteen differentials although they had shown different reactions on the above mentioned three varieties of barley These tests were confirmed twice and the result is given in Table II.

It is evident, therefore, that the two forms cannot be isolated from each other on the basis of reactions of the differentials selected by Levine and Cherewick (1952). The reactions given in table II do not tally with any of the 52 races described by them. This race, however, shows a close resemblance to race 2 of Cherewick and Levine in its reactions to all the "Critical differentials" except that the reactions of differential varieties Quinn and Letchtaler show some minor differences. Perhaps under identical conditions the differences might not be so significant as to call it a

new race distinct from race 2. Considering the reactions of all the differentials, to the five samples, it can be safely concluded that so far only one race has been picked up in this country. However, there are two forms of the race which are quite distinct from one another as regards to their reactions on certain varieties other than differentials viz. B. 4, Afg-12 and Afg. 9-12 (Table I) and hence these can be called as biotypes of the same race. The race (Form 1) has been provisionally designated as race H_1 and the other, its biotype as H_1-A.

TABLE - II. Reactions of differential varieties to five isolates of *Puccinia hordei*.

Name of differential hosts	Speciale	Reka	Sudan	Bolivia	Oderbruker	Quinn	Egypt—4	Gold	Letchtaler	Cruzat	Chilean D	Club mariout	Samaria	Berg	Austral	Kinver	Local (control)
Form 1 (Isolates 1 & 2 of Table 1)	4	0;	4	0;-1	4	0	3-4	0;	0;	3	3	3	3-4	3-4	1-2	4	4
Form 2. (Isolates 3, 4 & 5 of Table I)	4	0;	4	0;-1	3-4	0	3-4	0;	0;	3	3	3	3-4	3-4	2	4	4

INCUBATION PERIOD: The incubation period of the rust under glasshouse conditions at Delhi was found to be 7-8 days. At Simla it was found to be 8 to 9 days during summer and upto 11 days in winter.

COLLATERAL HOSTS : The study of grasses in relation to the cereal rusts is important in investigating the mode of perpetuation of the fungus as several grasses are known to harbour them. Their role as collateral hosts in nature, however, depends on many factors particularly, their habit and habitat. Waterhouse (1927 & 1929) and Mains (1930) found that a large number of species of *Hordeum* were susceptible to the rust in Australia and America. Natural infection of the rust on *Hordeum murinum*, *H. maritimum* and *H. secalinum* obtained by D'oliviera from Portugal proved to be of *P. anomala* (*P. hordei*).

During the present studies 48 species of cereals and grasses belonging to 24 genera were tested with a mixture of the isolates and the data are summarised in Table III.

TABLE - III Reactions of barley varieties and grass species to *P. hordei*

Name of species	Infection type
1. *Hordeum distichon* var. I.W. 66 ...	3C
2. *H. distichon* var. C.155–14 ...	2 ?
3. *H. murinum*	0;–1
4. *H. vulgare* var. N. P. 13. ...	3–4
5. *H. vulgare* var. NP. 21 ...	3–4
6. *Aegilops triuncialis* (E.C. 5049)	3
7. *A. ovata*	3–4C
8. *A. ventricose* (E. C. 5041) ...	3–4
9. *A. crassa* (E. C. 5041) ...	3
10. *A. triaristata*	3–4
11. *A. bicornis*	4
12. *A. squarrosa*	4

The remaining 36 grasses and cereals viz. *Aegilops cylindrica, A. biuncialis, A. caudata, A. speltoides, A. longissima, A. sharoneansis, Agropyron semicostatum, A. longearistatum, A. triticium, Cenchrus ciliaris, C. setigerus, Pennisetum polystachyum, P. pedicillatum, Panicum antidotale, Enteropogon monostachya, Chloris bourni, Dichanthium annulatum, Mellinis minutiflora, Eragrostis curvula, Bracharia brigantha, Bothriochloa inscalpta, Tricholaena rosea, Iseilema laxum, Briza minor, Vulpia myuros, Muehlenbergia hugelii, Heteropogon contortus, Digitaria* sp., *Urochloa* sp., *Secale cereale* (Rye), *Avena sativa, Triticum monococcum* var. *Einkorn, T. dicoccum* var. *Khapli, T. durum* var. *Mindum, T. compactum* var. *Little club* and *T. vulgare* var. *Agra local* were found to be immune.

Whereas *Aegilops ovata* is reported to be immune to the rust in Australia (Waterhouse, 1929), it got infected during these studies. On the other hand *Briza minor* reported to be slightly attacked by the Australian rust, was found to be immune. This difference may be due either to the different varieties of the grasses or different strains of the rust used.

Most of these grasses found to be immune to *Puccinia hordei* are indigenous to the hills and plains of India. It is worthwhile to mention here that no species of *Aegilops* is indigenous to India. Hence, as pointed out by Vasudeva *et al* (1953) indiscriminate introduction of grasses is fraught with danger and it is, therefore, absolutely essential to test all such materials, which are required to be introduced in this country, for their resistance to the rust, before their release.

SUMMARY

Germination studies of uredospores showed that the optimum range of temperature is between 16°–18°C. The uredospores remain viable upto 9 months if stored at 0°–5°C. and upto 4 months if stored at room temperatures (9°—14°C).

Five field collections from States of Delhi, Uttar Pradesh, and Madras were studied and it was demonstrated that the race in India is closely allied to race 2 described by Cherewick and Levine and that it has a biotype.

Forty eight species of cereals and grasses belonging to 24 genera were tested with the two forms of *P. hordei*, and 12 species belonging to *Hordeum* and *Aegilops* genera got infected by the rust.

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*Originals not seen.

SOME CERCOSPORA SPECIES FROM INDIA-I

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Since the establishment of the genus *Cercospora* by Fresenius in 1863, considerable amount of work has been done on this group of fungi. This is chiefly due to the fact that members of this group are conspicuous as plant parasites producing leafspots or blemishes, though in some cases they attack stem, blossom and fruit also, and cause economic losses. They are well adapted to different climatic conditions and affect a variety of plants. Conspicuous growth of the fungus and large size of spores have also been responsible for attracting the attention of those interested in the study of fungi. Several subdivisions in the past were proposed by different workers on the basis of colour of spores, conidiophores etc. but most of the mycologists now consider it more convenient to retain all the species in a common genus until more information on their perfect stage and other characters is available.

During the last several years, collection of fungi from different parts of the country has formed a part of our normal activities. The collections include specimens belonging to Cercosporae so that some of the specimens recently collected as well as those already available at Herb. Crypt. Ind. Orient., I.A.R.I. have been studied, which have resulted in establishing 19 new host records for India and two new species. These are reported in this paper.

Cercospora amorphophallii P. Henn., Hedwigia **41** : 147, 1902; Sacc. **18** : 611, 1906.

On living leaves of *Amorphophallus* sp. (Araceae), Lakshman Jhula, Rishikesh (U.P.), 7.10.1955, J. N. Kapoor.

The species under study agrees with Henning's species in its essential characters except that its conidia and conidiophores measure 21.6–72 x 2.7–4 μ. and 36–216 x 3.6–6.3 μ respectively, whereas in the original description conidial measurements are 40–65 x 2.5–3 μ. Furthermore, the shape of the conidia, has been described as oblong-cylindric while in the above collection the conidia are acicular thus agreeing with Chupp's (l.c. p. 56) description. The species, therefore, is being identified as *C. amorphophalli*. It is known to occur in Java, Japan, China, and Philippines. Another species, *C. amorphophalli* Pat. & Har. (Bul. Soc. Mycol. de France **24** : 15, 1909), which is a synonym of *C. chevalieri* Sacc., differs from the above species in having wider conidia and conidiophores and acicular to obclavate or cylindric conidia.

Cercospora buddleiae Yamamoto, Trans. Nat. Hist. Soc. Formosa **26** : 279, 1936.

On living leaves of *Buddleia* sp. (Loganiaceae), Simla, Punjab, March, 1950, R. L. Munjal.

The species agrees with Yamamoto's species except that its conidia and conidiophores measure 25.2–57.6 x 3.6–4.5 μ, and 36–115 x 3.6–5.4μ respectively whereas in the original description the conidiophores upto 119μ long and 6 μ wide and the conidia measuring upto 72 μ long and 5 μ broad are recorded. This species is already reported from Formosa and Japan.

Cercospora cavarae P. & D. Sacc., Syll. Fung. **16** : 1069, 1902.

On living leaves of *Glycyrrhiza glabra* L. (Leguminosae), I.A.R.I., New Delhi, 21–1–1950, M. A. Viswanath.

The species under study falls very close to Saccardo's species in its essential characters and is already reported from Sardinia, Turkestan, Astarkhan, Middle Asia. Another species of *Cercospora* namely *C. glycyrrhizae* (Savulescu & Sandu -Ville) Chupp (l.c.p. 308), which differs from the above in having definite infection spots, longer conidiophores and acicular, hyaline conidia, has also been described on this host.

Cercospora cotizensis Müller & Chupp, Ceiba **1** : 173, 1950; Chupp, 1953, p. 297.

Syn. *Cercospora crotalariae* Syd., Ann. Mycol. **28** : 208, 1930.

,, *crotalariae* Sawada, Taihoku Soc. Agr. and Forestry Jour. **7** : 118, 1942.

On living leaves of *Crotalaria* sp. (Leguminosae), Pusa, Bihar, 14–4–1913, I. U. Khan.

It is a distinct species from other Cercosporae known on this host genus in having effuse fruiting; stromata up to 36 μ; non-fasciculate to fasciculate, branched, conidiophores measuring 15–75 x 3–5 μ; Conidia obclavate, olivaceous brown and 25–80 x 3–5 μ in size. It agrees with *C. cotizensis* in all its characters except in the slightly bigger size of the stromata. This species is recorded from Venezuela, Formosa and Guatemala.

Cercospora crotalariae Sacc., Ann. Roy. Bot. Gardens, Peradeniya **3** : 2, 1906; Sacc. **22** : 129, 1913.

Syn. *Cercospora crotalariae-junceae* Sawada, Taihoku, Soc. Agr. and Forestry Jour. **7** : 27, 1942.

On living leaves of *Crotalaria juncea* L. (Leguminosae), Pusa, Bihar, 27–11–1906, E. J. Butler; of *C. gareensis* (Leguminosae), I.A.R.I. New Delhi, 28–10–1950, R. L. Munjal.

This species can be separated from *C. demetrioniana* Wint. (Hedwigia **23** : 170, 1884) recorded on this host genus which shows wider and longer, acicular conidia; and darker, broader conidiophores. In the above collec-

tion, spots are pale brown with definite margin; conidiophores measure 18–57 x 4–5.4 μ and conidia 39.6–90. x 3–4μ on *C. gareensis* and 55–105 x 3–5 μ on *C. juncea*. Previously the fungus has been reported from Ceylon and Formosa.

Cercospora cruciferarum Ell. & Ev., Jour. Mycol. **3** : 17, 1887; Sacc. **10** : 619, 1892.

On living leaves of *Raphanus sativus* L. (Cruciferae), State Agric. Farm, Kalimpong, West Bengal, 19–12–1957, S. P. Raychaudhuri.

There is another species, *C. atrogrisea* Ell. & Ev. (Proc. Acad. Nat. Sci. Phila. **45** : 464, 1863), reported on this host genus, which produces effuse patches on stems and pods and conidia are cylindric to acicular whereas the species under study produces definite infection spots; conidiophores are brown and measure 25.2–126 x 4.5–5.4 μ; and conidia are acicular, measuring 36–136.8 x 3.6–4.5 μ. Other characters alongwith those already given suggest that it is *C. cruciferarum*. Previously it is reported from U.S.A.

Cercospora davisii Ell. & Ev., Proc. Acad. Nat. Sci. Phila. **43** : 89, 1891; Sacc. **10** : 622, 1892.

On living leaves of *Melilotus parviflora* Desf.(Leguminosae), Pusa, Bihar, 25–2–1905, E. J. Butler.

Horsfall considered this species to be a synonym of *C. zebrina* (Mycologia **21** : 304, 1929) but Chupp (l.c.p. 301) after having studied all the species found on *Melilotus*, *Trifolium*, *Medicago* and *Lespedeza* is of the opinion that although all the species are very much alike, there are distinct morphologic differences among them which cannot be overlooked. The above collection shows irregular to linear brown spots, dark-brown stromata measuring upto 50.4 μ; brown conidiophores measuring 14.4–75.2 x 4–5.4 μ; and hyaline, obclavato-cylindric conidia, 39.6–75 x 4–4.5 μ in size. So far it is recorded from U.S. A. only.

Cercospora euphorbiaecola Atk., Cornell Univ. Bul. **3**(1): 41, 1897; Sacc. **14** : 1104, 1899.

On living leaves of *Euphorbia* sp. (Euphorbiaceae), Badamtam, Darjeeling, West Bengal, 2–9–1909, W. McRae.

In this collection the conidiophores measure 20–85 x 4–5 μ and conidia are upto 100 μ long and 4 μ wide. It is known from U.S.A., Minas Geraes, China and Uganda.

Cercospora jujubae Chowdhury, Indian J. Agric. Sci. **16** : 525, 1946.

On living leaves of *Zizyphus jujuba* Lam. (Rhamnaceae), Joginder Nagar, Punjab, 3–12–1957, H. S. Gill & V. S. Sharma.

This species is distinct from *C. zizyphi* Petch (Ann. Roy. Bot. Gardens Peradeniya **4** : 306, 1909) recorded on this host genus, in having broader

conidia and conidiophores and producing indefinite infection spots. The fungus has been previously recorded from Maulvibazar (now in Pakistan), Assam by Chowdhury (l.c.)

Carcospora malayensis Stev. &. Solheim, Mycologia **23** : 394, 1931.

On living leaves of *Hibiscus sabdariffa* L. (Malvaceae), Pusa. Bihar, 9–1–1911 M. Taslim.

This species is distinct from *C. brachypoda* Speg. (Annal. Soc. Cient. Argentina **13** : 28, 1882), known on this host genus, which shows hyaline cylindric conidia and short conidiophores whereas the above collection shows stromata upto 40 μ in diameter, conidiophores 45–100 x 5 μ and conidia measuring 60–125 x 3–5 μ which are acicular in shape.

Cercospora mississippiensis Tracy & Earle, Bull. Torrey Bot. Club **22** : 179, 1895; Sacc. **14** : 1105, 1899.

On living leaves of *Smilax* sp. (Liliaceae), Simla, Punjab, 16–9–1937, K. M. Dutt; Palampur, Kangra, Punjab, 1–9–1910, J. H. Mitter.

A number of species of *Cercospora* have been recorded on this host genus. The collection under study shows stromata upto 35 μ in diameter; conidiophores 21.2–70.6 x 4.4–5.2 μ and conidia 65–125 x 4–5 μ thus resembling very closely *C. mississippiensis*.

Cercospora osbeckiae sp. nov.

Maculae circulares vel subcirculares, rarae, dispersae, griseoalbidae in medio, nigro-brunneae ad margines, 2–4 mm. diam; fructificationes amphigenae; stromata fusce brunnea, subglobosa, 25.2–39.6μ diam.; fasciculi rari, divergentes; conidiophori olivaceo-brunnei, sparse septati, non ramosi, irregulares latitudine, angusti atque dilute colorati ad apices, raro geniculati, 10.8–28.8 x 4–5.4 μ; conidia hyalina, acicularia, septata, recta vel curva, truncata ad basin, acuta ad apicem, 54–144 x 2–4 μ.

Typus lectus in foliis viventibus *Osbeckiae stellatae* Wall. e familia Melastomaceearum, ad Chakung, in regione Sikkim, mense aprili anni 1957 a J. N. Kapoor et positus in Herb. Crypt. Ind. Orient, New Delhi sub numero accessionis 25916.

Cerocospora osbeckiae sp. nov.

Leaf spots circular to subcircular, few, scattered, grayish white centre with blackish brown margin, 2–4 mm. in diameter; fruiting amphigenous; stromata dark brown, subglobose, 25.2–39.6 μ in diameter; fascicles few, divergent; conidiophores olivaceous brown, sparingly septate, not branched, irregular in width, narrow and dilute coloured towards the tip, rarely geniculate, 10.8–28.8 x 4–5.4 μ; conidia hyaline, acicular, septate, straight to curved, base truncate, tip acute, 54–144 x 2–4 μ.

On living leaves of *Osbeckia stellata* Wall. (Melastomaceae), Chakung, Sikkim, April 1957, J. N. Kapoor, Type deposited at Herb. Crypt. Ind.

Orient, New Delhi. (Accession No. **25916**).

This species is distinct from all the Cercosporae recorded on the members of Melastomaceae to which *Osbeckia* belongs, and is, therefore, being described as a new species.

Cercospora pallidissima Chupp, A Monograph of the Fungus Genus Cercospora, p. 350, 1953.

On living leaves of *Smilax aspera* L. (Liliaceae), Mandi,(H. P.); 29–5–1955, L. M. Joshi.

The above collection possesses globose stromata which measure 39.6–72 μ in diameter; conidiophores are short and 10.8–32 x 3.6–5.4 μ and conidia are subhyaline to pale and measure 28.8–75.6 x 3.6–5 μ. So far it has been known only from Brazil, the type locality of this species.

Cerocospora petuniae (Saito) Muller & Chupp, Arch. Inst. Biol. Veg. Rio de Janeiro **3** : 96, 1936; Chupp, 1953, p. 546.

Syn. *Cercosporina petuniae* Saito, Trans. Totteri Soc. Agr. Sci. **3** : 271, 1931. On living leaves of *Petunia* sp. (Solanaceae), Punjab Univ. College, Hoshiarpur, Punjab, 27–5–1955, B. S. Bajaj.

The above collection on examination was found to be very close to *C. petuniae*, except that the stromata are slightly better developed measuring upto 39.6 μ in diameter. It is also recorded from Mines Geraes, Oklahoma, Wisconsin, Guatemala and Japan.

Cercospora psoraleae Ray, Mycologia **33** : 176, 1941.
Syn. *Cercospora psoraleae* Petrak, Sydowia **4** : 572, 1950.

„ *psoraleae-bituminoae* Savul. & Sandu, Annal. Acad. Romane Mem. Sect. Stuntif Ser. III, **15** : 484, 1941.

Leaf spots circular to irregular, with grayish centre and blackish-brown margin; epiphyllous, sometimes amphigenous; stromata lacking or slight; fascicles divergent, 3–15 stalks; conidiophores fasciculate, simple, septate, geniculate, dark brown, paler towards the tip, not branched, 28.8–126 x 3.6–5.4 μ; conidia hyaline, acicular, septate, straight to slightly curved, 21.6–72 x 2–3.6 μ.

On living leaves of *Cyamopsis tetragonoloba* Taub. (Leguminosae), I.A.R.I., New Delhi, 28–10–1950, A. N. Nagraj.

Cercospora pycnicola sp. nov.

Maculae circulares vel parum irregulares, dispersae, singulae, diametientes 0.5–3 mm, mediate brunnea ad medium, rubro-nigrae ad margines elevatos; fructificationes raro amphigenae, sed praesertim hypophyllae; fasciculi densi, divergentes; stromata nulla vel tenuia; conidio-

phori olivaceo-brunnei, pallidiores ad apices, flexuosi, sparse ramosi, 0–3 moderate geniculati, septati, recti vel curvi, irregulares latitudine, sporarum cicatrice prominente, 17.65–88.25 x 3.5–5.29 μ; conidia pallide olivaceo-brunnea, cylindrico-obclavata, multiseptata, recta vel curva, vacuolata, paulisper angustata supra, apice hebete, basi obconice truncata, 10.6–105.9 x 3.5–5.3 μ. Status pycnidialis: pycnia pallide brunnea, subepidermalia, in textus immersa, dispersa, ostiolata, parietibus tenuibus praedita, 59–72 x 42–60 μ conidiophori hyalini, uni–cellulati paulisper acuti supra, cylindrici, 15–20 x 2 μ; conidia hyalina, semel cellulata, ovalia vel ovata, 2–4 x 2–3 μ; conidiophori *Cercosporae* apparent circum ostiolum pycnii.

Typus lectus in foliis viventibus *Capparidis sepiariae* Linn. e familia Capparidacearum, ad Mandir Lane, in urbe New Delhi, die 29 julii anni 1954 a R. L. Munjal et positus in Herb. Crypt. Ind. Orient. New Delhi sub accessionis numero 25917.

Cercospora pycnicola sp. nov.

Leaf spots circular to slightly irregular, scattered, single, 0.5–3 mm. in diameter, medium brown centre with reddish black, raised margin; fruiting chiefly hypophyllous, rarely amphigenous; fascicles dense, divergent; stromata none or slight; conidiophores olivaceous brown, tip dilutely coloured, flexuous, sparingly branched, 0–3 mildly geniculate, septate, straight to curved, irregular in width, spore scar prominent, 17.65–88.25 x 3.5–5.29 μ; conidia pale olivaceous brown, cylindro-obclavate, multiseptate, straight to curved, vacuolate, slightly narrowed above, tip blunt, base obconically truncate, 10.6–105.9 x 3.5–5.3 μ.

Pycnial Stage: Pycnia, light brown, sub-epidermal, immersed in tissues, scattered, ostiolate, thin, walled 59–72 x 42–60 μ; conidiophores hyaline, single celled, slightly pointed above, cylindrical, 15–20 x 2 μ; conidia hyaline, single celled, oval to ovate, 2–4 x 2–3 μ; conidiophores of *Cercospora* appear around the ostiole of the pycnium.

On living leaves of *Capparis sepiaria* L. (Capparidaceae) Mandir Lane, New Delhi, 29–7–1954, R. L. Munjal, Type, deposited at Herb. Crypt. Ind. Orient. New Delhi. (Accession No. 25917).

An examination of the material revealed the presence of minute hyaline, single celled spores borne in pycnidia as well as typical fructifications of *Cercospora*. These Cercospora fructifications in all cases were borne on light-brown coloured pycnidia. Several *Cercospora* species are known to have *Mycosphaerella* as their ascigerous stage. The pycnial stage observed here appears to be a spermatial stage.

Three species of *Cercospora* with coloured conidia have been recorded on Capparidaceae, namely *C. capparidicola* Hansford & Thirum. (Farlowia 3 : 307, 1948), *C. conspicua* Earle (N. Y. Gard. Bul. 3 : 312, 1905) and *C. tovariae* Chupp & Mueller (Bol. Soc. Venez. Cient. Nat. 8 (52) : 58, 1942). The species under study differs from all the three by having definite infection spots. *C. capparidicola* is distinct on account of its wider conidiophores and conidia, whereas *C. conspicua* has amphigenous fructifications,

slightly narrower conidiophores and conidia; and *C. tovariae*, shows darker crooked conidiophores and cylindric conidia. Because of the consistent association of spermatial stage alongwith conidial stage observed in the present case, the name *C. pycnicola* has been proposed.

Cercospora riachueli Speg., Annal. Soc. Cient. Argentina **10** : 38, 1880; Sacc. **4** : 458. 1886.

Syn. *Cercospora horiana* Togashi & Katsuki, Sci. Repts. Yokohama Nat. Univ. Sect. II. **1** : 4, 1952.

On living leaves of *Vitis trifolia* L. (Vitaceae), Ridge Road, New Delhi, 17–10–1955, R. L. Munjal.

The above collection shows stromata measuring 36–64.6 μ conidiophores simple, septate, without geniculation, measuring 18–86.4 x 3.6–5μ ; conidia 28.8–129.6 x 4.5–6.3 μ. This species is already known from Argentina, Japan and Puerto Rico.

Cercospora rubro-tincta Ell. & Ev., Jour. Mycol. **3** : 20, 1887; Sacc. **10** : 643, 1892.

Syn. *Cercospora consbrina* Ell. & Ev., Jour. Mycol. **3** : 19, 1887.

,, *guliana* Sacc., Annal. Mycol. **11** : 565, 1913.

,, *amygdali* Riza, Bul. Soc. Mycol. France **36**: 191, 1920.

On living leaves of *Prunus amygdalus* Batsch. (Rosaceae), Partap Farm, Srinagar, Kashmir, June, 1944, A. Khan.

The above collection matches very well with *C. rubro-tincta* in all its details except that the conidiophores are 14–36 x 2.6–5 μ and conidia measure 32–54 x 3.6–5μ and are typically cylindro-obclavate.

C. prunicola Ell. & Ev. (Jour. Mycol. **3** : 17, 1887) also recorded on this host genus can be distinguished in having much shorter and slightly thinner conidiophores.

Cercospora ubi Racib., Bot. Inst. Buitenzorg, Batavia. Par. Algen u. Pilze Javas **3** : 39, 1900; Sacc. **16** : 1073, 1902.

Syn. *Cercospora brasiliensis* Averna, Biol. Agr. Sao. Paulo xviii. A. **7** : 580, 1917.

On living leaves of *Dioscorea sativa* L. (Dioscoreaceae), I.A.R.I., New Delhi, 21–1–1956, Gian Singh and Ved Prakash.

The previous record of this species from India by Sydow and McRae, (Ann. Crypt. Exot. II : 270, 1929) was from Chittagong, which is now in Pakistan.

Cercospora voandzeiae Bouriquet, Encycl. Mycolog. **12** : 357, 1946.

On living leaves of *Voandzeia subterranea* Thou. (Leguminosae), I.A.R.I., New Delhi, 28–10–1950, R. L. Munjal.

The above collection agrees fully with the original description of the Type species except that the leaf spots are at first indistinct but gradually become irregularly circular with whitish centre and tan coloured margin. The species is already known from Madgascar and Uganda.

Cercospora zebrina Pass., Hedwigia **16**: 124, 1877; Sacc. **4**: 437, 1886.

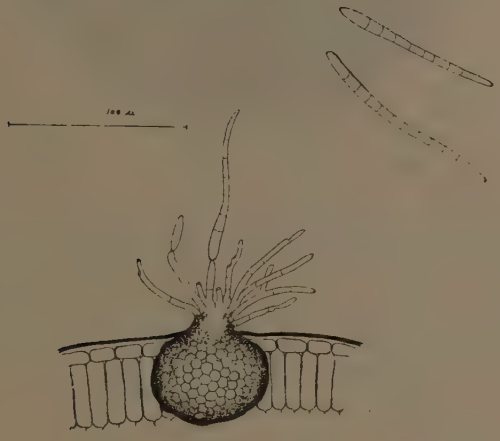
Syn. *Cercospora helvola* Sacc., Michelia **2**: 556, 1882.

,, *stolziana* Magnus, Die Pilze von Tirol. (etc.) p. 558. 1905.

,, *helvola* var. *zebrina* Ferraris, Fl. Ital. Crypt. **1** (8): 423.

On *Trifolium alexandrinum* L. (Leguminosae), Pusa, Bihar, 5-5-1917, E. J. Butler.

The above collection on examination showed small stromata; with conidiophores measuring 22–106 x 3.5–5.3 μ. The conidia are acicular hyaline and 43–79 x 2–5.4 μ in size.



Cercospora pycnicola

Cercospora osbeckiae

Cercospora pycnicola

The authors wish to place on record their grateful thanks to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology & Joint Director, Indian Agricultural Research Institute, for his keen interest, helpful criticism and valuable suggestions throughout this work. Our thanks are also due to Rev. Father. Santapau for rendering latin diagnosis of the new species and to Mr. J. N. Kapoor, Herbarium Keeper, I.A.R.I., for making available some of his collections for our study and for assistance in the determination of some of them.

Division of Mycology & Plant Pathology,
I.A.R.I., New Delhi.

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Chupp, C. (1953) A Monograph of the Fungus Genus *Cercospora*. *Ithaca, New York*, 667pp.

Solheim, W. G. (1929) Morphological studies of the Genus *Cercospora* III. *Biol. Mono.* **12**(1), 84 pp.

SOME CERCOSPORA SPECIES FROM INDIA - II

R. L. Munjal, G. Lall and B. L. Chona

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The present paper gives an account of 9 species of *Cercospora* of which two are new and seven are either new records or new host records for India. The specimens have been deposited in Herb. Crypt. Ind. Orient., I.A.R.I., New Delhi and their accession numbers are indicated in the text.

Cercospora brunkii Ell. & Galloway, Jour. Mycol. **6** : 33, 1889; Sacc., Syll. Fung. **10** : 620, 1892.

Syn. *Cercospora pelargonii* Mendoza, Philipp. Jour. Sci. **75** : 176, 1941.

On living leaves of *Pelargonium* sp. (Geraniaceae), Der (Bombay), August, 1918, L. J. Sedgwick (HC10 No. 25991).

Leaf spots circular or oval, brown, surrounded by raised, darker margin; fruiting amphigenous; stromata slight; fascicles not dense; conidiophores olivaceous brown, septate, not branched, sometime geniculate, 42–136 x 3.5 – 5.0 μ; conidia acicular to obclavate, straight to curved, hyaline, septate, 35.00–156 x 3.5–4.5 μ.

Cercospora caricis Oud., Nederl. Kruidk. Archief II. **6** : 59, 1892; Sacc., Syll. Fung. **25** : 900, 1931 as *Cercosporina*; Govindu & Thirum., Sydowia **8** : 224, 1954.

Syn. *Cercospora caricina* Ell. & Dearness, Proc. Can. Inst. N. S. Part 3, **1** : 91, 1897.

Cercospora microstigma Sacc., Ann. Mycol. **10** : 315, 1912.

Cercospora caricis Dearness & House, N. Y. State Mus. Bul. **188**: 29, 1916.

On living leaves of *Cyperus subcapitatus* Clarke (Cyperaceae), Pusa (Bihar), 22–3–1906, Inayat Khan (HC10 No. 25995).

Leaf spots pale to dark brown, definite amphigenous; stromata small; fascicles few; conidiophores olivaceous brown, septation indistinct, geniculate, not branched, straight to curved, 11-18 x 3.5 – 5μ; conidia hyaline, obclavato-cylindric, septate, straight or slightly curved, 21–95 x 2.5 – 4μ.

This species was earlier reported by Govindu & Thirum. (l.c.) on *Eleocharis fistulosa* (Cyperaceae) from South India.

Cercospora coccíniae sp. nov.

Leaf spots circular to irregular, 0.5–6 mm. in diameter, scattered or sometimes confluent, grayish-white centre with black margin on the under-surface and white centre with reddish brown, raised margin on the upper side; fruiting amphigenous; fascicles few, spreading; stromata slight, brown; conidiophores fasciculate, simple, septation indistinct, geniculate, straight to curved, olivaceous-brown, tip dilutely coloured, spore-scar prominent, some bulbous at the base, 14–42 x 4.4–7μ; conidia hyaline, obclavato-cylindric, straight to curved, tapering above, base obconically truncate, tip subacute, 35–117 x 4.4–6μ.

On living leaves of *Coccinia indica* Wt. & Arn. (Cucurbitaceae), 4–9–54, Karnal (Punjab), B. S. Bajaj. Type (HC10 No. 26098).

Cercospora coccíniae sp. nov.

Foliorum maculae circulares vel irregulares, 0.5–6 mm. diam., dispersae vel aliquando confluentes, griseo-albidae in medio, marginibus nigris in pagina inferiore, in superiore vero albae in medio, marginibus rubro-brunneis; fructificatio amphigena; fasciculi rari, patentes; stromata brunnea; conidiophori fasciculati, simplices, geniculati, recti vel curvi, olivaceo brunnei, septis indistinctis, apice dilute colorato, sporarum cicatrice eminente, nonnulli bulbosi ad basin, 14-42 x 4.4–7μ; conidia hyalina, obclavato-cylindrica, recta vel curva, fastigata supra, truncata obconice ad basin, apice subacuto, 35–117 x 4.4–6μ.

Lectus in foliis viventibus *Cocciniae indicae* Wt. & Arn. e Cucurbitaceis die 4 septembris anni 1954 ad Karnal in region Punjab a B.S. Bajaj. Typus. (HC10 No. 26098).

Cercospora latens Ell. & Ev., Jour. Mycol. 4 : 3, 1888; Sacc., Syll. Fung. **10** : 641, 1892.

Syn. *Cercospora lespedezae* Ell. & Dearness, Proc. Can. Inst. N. S. part, 3, **1** : 91, 1897.

On living leaves of *Psoralae corylifolia* L. (Leguminosae), Pusa and Samastipur (Bihar), 29–12–1906, Inayat Khan (HC10 No. 25994).

Leaf spots irregular, effuse, ferruginous in colour; fruiting mostly on the upper surface but sometimes amphigenous; stromata small; fascicles dense; conidiophores pale olivaceous-brown, rarely septate, not branched 22–43 x 3.5–5 μ; conidia obclavate to cylindric, subhyaline to pale, straight or slightly curved, base obconically truncate, 40–86 x 3.5–5.5μ.

Cercospora mori Hara, Jour. Sericult. Assoc. Japan **27**: 227, 1918; Chupp A monograph of the fungus genus *Cercospora*, 1953.

Syn. *Cercosporamori* Marchal & Steyaert, Bul. Soc. Roy. bot. de Belg. **61**(n.s.) **11** : 166, 1929

On living leaves of *Morus alba* L. (Moraceae), I.A.R.I., New Delhi (Delhi), Nov. 1958, R. L. Munjal (HC10 No. 26097).

Leaf spots indistinct at first but later olivaceous to dark coloured on the undersurface due to the effuse growth of the conidia and conidiophores with a corresponding yellow discoloration on the upper surface changing to brown colour; Colonies numerous, small scattered, later coalescing and covering the entire leaf; stromata none or a few brown cells with 4–7 spreading conidiophores in loose fascicles; conidiophores pale-olivaceous to olivaceous, not branched, rarely 2–3 septate, slightly clavate or cylindric, with conic or rounded tip, flexuous, 1–4 geniculate, 21–35 x 3–7μ; conidia concolorous with the conidiophores, obclavate or short ones almost cylindric, some curved, 3–7 septate, mostly 15–30 x 3–5μ, rarely a few 6–8 μ broad.

This fungus resembles *Cercospora mori* Hara, as described by Chupp (l.c.p. 399). In its growth habits and the shape of conidia, this fungus is very close to *Cladosporium* Link, but has conidiophores typical of *Cercospora*. This appears to be a border-line species between *Cercospora*. and *Cladosporium*. The species described by Marchal and Steyaert (l.c.) as *C. mori* on *Morus* sp., from Belgian Congo, agrees with the fungus described here, except that the conidia and conidiophores are smaller in our specimens but that being a later homonym, is not valid.

Cercospora oculata Ell. & Kellerm., Bull. Torrey Bot. Cl., **11** : 116, 1884; Sacc., Syll. Fung. **4** : 443, 1888.

On living leaves of *Vernonia cinerea* Less. (Compositae), I.A.R.I., New Delhi (Delhi), 28–10–1956, R. L. Munjal (HC10 No. 25992).

Leaf spots zonate, brown, surrounded by narrow, raised, black margin, sometimes confluent; fruiting amphigenous; stromata small, fascicles mostly dense; conidiophores brown, septate, rarely geniculate, not branched, 11–67 x 3.5–5 μ; conidia cylindric, pale olivaceous, straight or slightly curved, septate, 35–76 x 3.5–5μ.

The species is distinct from *Cercospora oculata* Ell. & Kellerm. var. *indica* Govindu & Thirum. (Sydowia **9** : 223, 1955), reported from India on *Vernonia bourneana* which has thinner conidia and bifurcate conidiophores.

Cercorspora pericampyli sp. nov.

Leaf spots indefinite on the upper side of the leaf appearing as brownish discolourations; on the lower side effuse patches, dirty-black in colour, irregular, scattered or confluent, vein-limited; fructifications chiefly hypophyllous, but rarely amphigenous; stromata small, olivaceous-brown; fascicles dense; conidiophores fasciculate, simple, straight or slightly bent, olivaceous-brown, septation, geniculation and branching lacking or indistinct, tip pointed, truncate or sometimes swollen, 10–28 x 2.0–3.5μ; mycelium thin, septate, intracellular; conidia subhyaline to pale, 2–8 septate, cylindro-obclavate, smaller almost cylindric, tapering above 21–69 x 3–7 μ, base obconically truncate, tip subobtuse.

On living leaves of *Pericampylus* sp. (Menispermaceae), 15–4–1916, Pusa (Bihar), N. C. Sen Gupta, Type (HC10 No. 26099).

Cercospora pericampyli sp. nov.

Foliorum maculae indefinitae in pagina superiore, discolorationes brunneae adsunt, in inferiore vero maculae effusae, sordide nigrae, irregulares, dispersae vel confluentes, limitatae nervis; fructificatio ut plurimum hypophylla, raro amphigena; stromata parva, olivaceo-brunnea; fasciculi densi; conidiophori fasciculati, simplices, recti vel tenuiter curvati, olivaceo-brunnei, septis, geniculis et ramis aut nullis aut indistinctis, apice acuto, truncato vel nonnumquam tumido, 10–28 x 2.0–3.5μ; mycelium tenue, septatum, intracellulare; conidia subhyalina vel pallida, bis ad octies septata, cylindrico-obclavata, minora quidem fere cylindrica, fastigata supra, 21–69x3–7μ, truncata obconice ad basin, subobtusa ad apiceum.

In folus viventibus *Pericampyli* sp. e Menispermaceis, die 15 aprilis anni 1916 ad Pusa in regione Bihar, a N.C. Sen Gupta Typus (HC10 No. 26099).

Cercospora sidaecola Ell. & Ev., Jour. Mycol. **5** : 72, 1889.

Syn. *Cercospora densissima* Speg., Anal. Mus. Nac. B. Aires. Ser. 2, **3**: 241, 1889.

On living leaves of *Sida spinosa* L. (Malvaceae), Faridabad (Punjab), 29–8–1958, J. N. Kapoor (HC10 No. 25993).

Leaf spots indistinct; fruiting in dark olivaceous effuse patches mostly on lower leaf surface, confluent, stromata absent, mostly non-fasciculate; conidiophores dark brown uniform in colour and width, septate, geniculate, rarely branched, 36–216 x 3.5-4 μ; conidia hyaline, acicular to obclavate, septate, straight to curved, 21–100 x 3–4.5μ.

Cercospora withaniae H. & P. Syd., Ann. Mycol. **10** : 444 1912; Sacc., Syll. Fung. **25** : 891, 1931.

On living leaves of *Withania somnifera* Dun. (Solanaceae), I. A. R. I. New Delhi (Delhi), 29–9–1958, G. Lall, (HC10 No. 26100).

Leaf spots indistinct, irregular yellowish to brown discolorations on the upper surface of the leaf; fructifications hypophyllous, confluent, gregarious, effuse, dark; stromata small, dark-brown; fascicles dense; conidiophores pale to olivaceous brown, septate, branched, curved, tortuous 14–79 x 3.5–5.5μ; conidia pale olivaceous-brown, obclavate-cylindric, straight to curved, septate 29–90 x 3.5–5.5μ.

The earlier record of this species by Mundkur & Ahmed (I.M.I., Mycol. paper No. **18** : 10, 1946) is from a locality which is now in Pakistan.

Fig. 2. *Cercospora mori*, Conidiophores and conidia

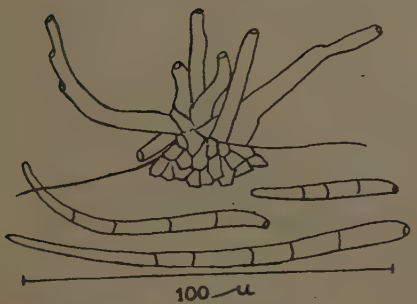

Fig. 1. *Cercospora cocciniae*. Conidiophores and conidia

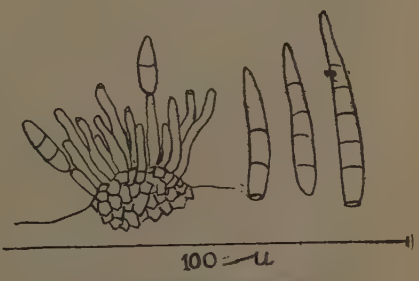

Fig. 3. *Cercospora pericampyli*, Conidiophores and conidia

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his keen interest, helpful criticism and encouragement. We are also indebted to our colleague, Mr. J. N. Kapoor, Herbarium Keeper, for his willing co-operation in the determination of some species and to Rev. Fr. Dr. H. Santapau for rendering the latin diagnosis of the new species.

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A STUDY ON THE PRESERVATION OF FUNGAL CULTURES BY THE MINERAL OIL METHOD

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The two chief problems of any large culture collection of fungi, as of other micro-organisms, are (1) preservation of cultures in a viable condition for extended periods of time, so that attention may be directed to other work than routine maintenance of the organisms, and (2) ensuring the continuation, in an unimpaired condition, of the morphological, pathogenic and biochemical characteristics of the original cultures. In highly variable fungi mere reduction in the frequency of subculturing ensures the maintenance of such characteristics to a certain extent, though the converse has also been noted in phytopathological practice, namely, the need for frequent subculturing accompanied by single sporing, in order to ensure the pathogenicity of the isolates. Simple storage in the cold after the organism has fully grown, with or without some method of preventing the dehydration of the medium (e.g., use of screw-capped bottles, paraffin- coated paper and plastic seals of one type or the other, and hermetical sealing**) is known to keep certain fungi viable over long periods. However, such methods are unreliable in many cases. More reliable methods are available, namely, lyophilization, maintenance under mineral oil, preservation in sterilized sand (Galloway, 1936), soil or vegetable substrata (Thom and Raper, 1945; Bakerspiegel, 1953, 1954; Atkinson, 1954; Backus and Stauffer, 1955), storage after spray- drying (Mazur and Weston, 1949; Mazur and Weston, 1956) or simple drying (Bedi, 1949, Rhodes 1950), or freezing (Purvis and Barnett, 1952; Carmichael, 1956). Among these, one or the other modifications of lyophilization (Wickerham and Andreasen, 1942; Raper and Alexander, 1945; Ledingham, 1947; Haskins and Anastasion, 1953; Mehrotra and Hesseltine, 1958) is to be preferred in the maintenance of molds and other fungi which produce firm-walled spores or other propagative cells capable of withstanding freeze-drying. However, where the method is attended by difficulties such as those mentioned by Atkin et al (1949), and for non-sporulating fungi, the mineral oil method holds great advantages as is evident from the papers of Buell and Weston (1947), Stebbins and Robbins (1949), and Fennel et al (1950), who have amply reviewed earlier literature on the subject and discussed the relative merits of different methods of preservation. Since then, the mineral oil method has received greater attention in several laboratories (Reischer, 1949; Wernham and Miller, 1949; Kelman, 1950; St. John Brooks, 1951; Chaulan, 1951; Ajello et al, 1951; Schulze, 1951; Graham, 1952; Fergus and Cole, 1955; Haynes, Wickerham and Hesseltine, 1955; Hartsell; 1956). The majority of these reports mention viabilities of about 2½ years in most cases, and of three to five years in certain cases (Weiss and Oteifa, 1953). Viability exceeding this

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** We have found that a hermetically sealed culture of *Monascus purpureus* stored in the cold was viable for about ten years, whereas a large number of other similarly preserved organisms had died within this period.

period has been reported by Henry (1947) for certain yeasts and by Hartsell (1956) for some strains of *Saccharomyces*.

In 1949 an effort was made in the Indian Type Culture Collection to apply the mineral oil method to a large number of fungi. The results of such tests carried out over a period of eight years are reported here.

Experimental Results. The methods and precautions followed were essentially the same as those described by Buell and Weston (1947). The mineral oil chiefly employed was 'Nujol', a colourless commercial product of Messrs. Fassett and Johnson Ltd., London. The cultures were grown on one of the appropriate maintenance media: Potato dextrose agar, Oat meal agar, Malt extract agar and Corn meal agar. In rapidly growing organisms the sterilized oil was added eight to ten days after culturing; in others, it was added three to four months later. After the addition of oil, the cultures were stored in the cold at a temperature of 10°–12°C. The cold storage unit failed a few times during the eight years period of the test, but such failures were only for short durations not exceeding some hours, or a day at the most.

The results of the tests conducted are given in Tables I and II. Viability tests were conducted at different intervals. The period of viability shown against each culture in Table I represents the time at which the viability test was made.

From the tabulated results it is seen that out of the 11 species of fungi belonging to the Phycomycetes that were tested, 10 were found to have lost viability within a period of 4 years. *Zygorhynchus macrocarpus* which was found viable after 7 years and 7 months is homothallic and its viability could be attributed to the zygotes that are formed readily in culture. It is probable that if mated cultures with zygotes of other heterothallic forms of the Mucorales are preserved in mineral oil, they may similarly remain viable for long periods. It is also observed that while certain yeasts viz., *Hansenula belgica*, *Saccharomyces cerevisiae* var. *cratericus*, and *Schizosaccharomyces pombe* were found viable after 7 years, several other related species such as *Debaryomyces tyrocola*, *Eremothecium ashbyii*, *Pichia farinosa*, and *Saccharomyces carlsbergensis* lost viability within a period of 4 years. The five species each of *Chaetomiums* and *Sordaria* tested were found viable for over 7 years. *Mycosphaerella rabiei* which had ceased to sporulate in transfer cultures was found viable in mineral oil when tested after over 7 years and sporulated abundantly on oat agar. The mineral oil method of preservation seems to be specially suited to fungi belonging to the Eubasidiomycetes where most of the fungi tested were found viable after 7 years. The results with *Fusaria* and several other plant pathogenic fungi were similarly encouraging. It is, however, interesting to note that both *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (Strain b) which produced apparently resistant sclerotia in culture were not viable when tested after 4 years.

We have observed slow but perceptible growth in several cultures over long periods of storage under oil at 20–28 °C. Because of this fact

we have always found it desirable to maintain oil preserved cultures at refrigerator temperature of 5–10 °C.

B. O. C. 120, a brown coloured mineral oil obtained from M/s Burma Shell Oil Co., was also used in some tests and the results were almost identical with those obtained with Nujol.

Discussion. Though large numbers of fungi belonging to all the major classes can be preserved by the oil method, we have observed that viability under oil is not necessarily uniform, even in closely allied organisms. Such an observation has also been made by Fennel et al (1950) who found that in oil tests over 2 to 2½ years, the viability of certain members of Mucorales was excellent, but of others of the same order poor or negative; the percentage of non-viable cultures under oil was much less than on ordinary agar slants, but still fairly high. Such differences among closely related organisms or strains of the same species are also obvious from the data presented.

The literature on mineral oil preservation tends to suggest a very general applicability of the method in conserving cultures of fungi. Our experience shows that this is undoubtedly true where viabilities of about a year or two are aimed at, but in large culture collections where preservation over still longer periods is desired, it would be better to supplement oil-preservation with other known methods until more data are available about their behaviuor under oil. We do not imply here that the method is less advantageous than others, for wide divergence in the length of viability is also noted in lyophilization (Coffey 1952), which is one of the most widely used procedures at the present time. In fact, where tests have shown unimpaired viability over several years, as in this and similar studies, the oil method is to be preferred because of its simplicity.

One disadvantage of the oil method as reported here and, generally in the literature, is the large amount of storage space needed for the cultures and the necessity to maintain the tubes upright. This can be largely obviated in sporulating fungi if suspensions of the spores in oil can be kept in small, sealed ampoules. We have had insufficient experience of this method to either recommend or reject it.

Summary

The mineral oil preservation method has been applied to many species of fungi in the Indian type Culture Collection and, in conformity with data available in literature, found to be of great utility in maintaining cultures for long periods.

Though viabilities of upto eight years have been recorded in many fungi, it has also been observed that such extended viability is not uniform even in closely related fungi.

ACKNOWLEDGMENTS. The authors take the opportunity to express their appreciative thanks to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute for the interest he has taken and the encouragement he has provided for this work.

TABLE I. Species of fungi* found to be viable under mineral oil in a eight year test.

Organism	Found viable at Years	Months	
PHYCOMYCETES			
Zygorhynchus macrocarpus Ling	7	7	
ASCOMYCETES			
Arachniotus aureus (Ed.) Schroet.	7	10	
Chaetomium affine Cda.	7	1	
,, cochlioides Palliser	7	1	
,, elatum Kunze et Schmidt	7	1	
,, globosum Kunze	7	1	
,, convolutum Chivers	7	1	
Gymnoascus reesii Baranetzki	7	4	
Hansenula belgica (Lindner) Syd.	7	7	
Mycosphaerella rabiei (Pass.) Labr.	7	9	
Neurospora sitophila Shear et B.O. Dodge	8	0	
Saccharomyces cerevisiae Hansen var. cratericus (Lindner) Lafar	7	9	
Schizosaccharomyces pombe Lindner	7	3	
Sordaria inaequalis Cain	7	10	
,, curvispora Cain	7	10	
,, setosa Wint.	7	10	
,, humana (Fckl.) Wint.	7	10	
,, curvicolla Wint.	7	10	
BASIDIOMCYETES			
Collybia velutipes (Curt.) Lond.	7	3	
Cyathus striatus (Huds.) Pers.	7	5	
Daedalea flavida Lev.	5	2	
Fomes lividus Kalchb.	7	1	
,, pachyfloeus Pat.	7	3	
,, roseus (Alb. et Schw.) Cke.	7	3	
Ganoderma applanatum (Pers.) Pat.	7	3	
Lenzites striata (Sw.) Fr.	7	3	
Melanopsichium eleusinis (Kulkarni) Mundkur et Thirumalachar	5	9	Dead at 7 years 10 months.
Pellicularia salmonicolor (Berk. et Br.) Dastur	5	2	Dead at 7 years 3 months.

Organism	Found viable at Years	Months	
Polyporus agaricus Berk.	4	10	
,, gilvus Schw. forma gilvoides	7	3	
,, gilvus Schw.	7	3	
,, ostereiformis Berk.	7	3	
Polystictus affinis	5	2	Dead at 7 years.
,, tabacinus Mont.	7	3	
,, velutinus Fr.	7	3	
,, sanguineus (L.) Mey	7	3	
Psalliota campestris (L.) Fr.	8	1	
Schizophyllum commune Fr.	7	1	
Sphacelotheca sorghi (LK.) Clinton	5	9	Dead at 7 years 10 months.
Stereum fuscum (Schrad.) Quel.	7	8	
Tolyposporium ehrenbergii (Keuhn) Pat.	7	10	
Trametes cingulata Berk.	7	3	
,, cinnabarinus (Jacq.) Fr.	7	8	
,, pini (Brot). Fr.	7	7	
FUNGI IMPERFECTI			
Aspergillus clavatus Desm.	8	0	
,, flavipes (Bain. et Sart.) Thom et Church	7	11	
,, flavus Link	7	9	
,, japonicus Saito	5	9	
Alternaria brassicae (Berk.) Sacc.	7	9	
,, capsici-annui Savul. et Sandu	6	2	
Botrytis allii Munn.	6	1	
Cercospora cruenta Sacc.	6	11	
Curvularia lunata (Wakker) Beodijn	4	1	
Fusarium anguioides Sherb.	4	1	
,, avenaceum (Fr.) Sacc.	6	2	
,, bostrycoides Wr. et Rkg.	8	1	
,, bulbigenum Cke. et Mass.	4	1	
,, cubense E. F. Sm.	4	1	
,, culmorum (W. Sm.) Sacc.	4	1	
,, diversisporum Sherb.	4	1	
,, fructigenum Fr.	7	11	
,, lini Bolley	5	10	
,, moniliforme Sheld. var. majus Wr. et Rkg.	4	1	
,, orthoceras App. et Wr.	7	5	
,, orthoceras var. ciceri Padwick	7	7	
,, semitectum Berk. et Rav.	4	1	
,, solani (Mart.) App. et Wr. var. Martii (App. et. Wr.) Wr.	6	2	
,, udum Butl.	5	10	
,, vasinfectum Atk.	8	1	

Organism	Found viable at			
	Years		Months	
Helminthosporium bicolor Mitra	6	—	1	
,, halodes Drechsler	7	—	4	
,, ,, var. tritici Mitra	5	—	4	
,, sativum P. K. et B.	7	—	5	
,, tetramera Zaleski	6	—	1	
Oospora lactis (Fres.) Sacc.	7	—	1	
Paecilomyces varioti Bain.	6	—	0	
Penicillium gladioli Machacek	6	—	0	
,, terlikowskii Zaleski	8	—	0	
Piricularia oryzae Cav.	5	—	2	Dead at 7 years 1 month.
Sclerotium rolfsii Sacc. (Strain a)	4	—	1	

TABLE II. Fungi* which were not viable at 4 years.

PHYCOMYCETES

Dicranophora fulva Schroet.
Isoachyla unispora Coker et Couch
Phycomyces blakesleeanus Burgeff
Pythium aristosporum Vanterpool
Pythium indigoferae Butler
Pythium periplocum Drechsler
Rhizopus nigricans Ehrenberg
Sporodinia grandis Link
Thamnidium elegans Link
Trachysphaera fructigena Tab. et Bunt.

ASCOMYCETES

Ashbya gossypii (Ashby et Nowell) Guill.
Claviceps purpurea (Fr.) Tul.
Debaryomyces tyrocola Konokotine
Eremothecium Ashbyii Guill.
Pichia farinosa (Lindner) Hans.
Saccharomyces carlsbergensis Hansen
Sclerotinia sclerotiorum (Lib.) Massee

BASIDIOMYCETES

Polystictus hirsutus (Wulf) Fr.
Lentinus subnudus Berk.

FUNGI IMPERFECTI

Achorion Schoenleinii (Libert) Remak
Botrytis cinerea Pers.
Candida guilliermondii (A. Cast.) Langeron et Guerra
Candida reukaufii (Grüss) Diddens and Lodder

Cercospora blumeae Thuem.
Cercospora hibisci Tracy and Earle
Cercospora indica Singh
Chlamydomyces palarum (Cke.) Mason
Sclerotium rolfsii Sacc. (Strain b)

(*The names of the fungi used are those under which they were deposited in the collection.)

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